Barts and The London

Queen Mary's School of Medicine and Dentistry

Reducing acute kidney injury in patients with chronic kidney disease undergoing cardiac surgery

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Thesis submitted for MD (Res)

University of London, 2013

This thesis is dedicated to the memory of Akhil Kapur. He was my mentor and my friend. He believed in me and gave me the chance to undertake this research. I hope this is a fitting tribute to him.

Abstract

Patients with chronic kidney disease (CKD) are a group with a markedly increased risk of adverse events following cardiac surgery. A particular problem for these patients is the development of post-operative acute kidney injury (AKI), which is associated with a significant increase in morbidity and mortality. Currently, there are no effective therapies proven to modify AKI in patients undergoing cardiac surgery.

This thesis has three parts. The first describes an analysis of the Barts Health NHS Trust cardiac surgical dataset. Specifically, outcomes of patients with CKD and AKI were examined. The second describes a randomized control trial that examined the effect of remote ischaemic preconditioning (RIPC) upon AKI and myocardial injury in patients with CKD undergoing coronary artery bypass graft surgery (CABG). The final part describes the development of a panel of AKI biomarkers to allow the accurate prediction of AKI in patients with CKD undergoing CABG.

The aims of this thesis were:

1. In our local cardiac surgical cohort,
    a. To assess the effects of CKD upon outcomes after CABG.
    b. To asses the prognostic importance of AKI after CABG.
2. To assess the potential for RIPC to reduce AKI and myocardial injury in patients with CKD undergoing CABG.
3. To investigate the diagnostic performance of serum and urine AKI biomarkers in a population of patients with CKD undergoing CABG.

Analysis of the Barts Health NHS Trust cardiac surgical dataset confirmed that patients with CKD account for almost one-third of patients undergoing CABG. However, these patients account for a disproportionate two-thirds of all early mortality. CKD was also independently associated with late mortality after CABG. AKI was common in these patients. AKI was associated with late mortality even after accounting for pre-operative comorbidity and surgical complexity.

In the randomized control trial, RIPC showed no effect upon the incidence of AKI or myocardial injury in the. 86 patients with CKD recruited. Secondary analysis of serum and

urine biomarkers collected found change in serum cystatin C and NGAL as impressive predictors of AKI in patients with CKD. They allowed accurate early prediction of AKI more than 24 hours before diagnosis was possible using serum creatinine.

Acknowledgements

I am indebted to my supervisor Prof Magdi Yaqoob for taking me under his wing when Akhil became unwell. I could not have completed this thesis without him.

At William Harvey Research Institute I would like to thank Dr Steve Harwood his patience and support when teaching me scientific methodology.

At the London Chest Hospital, I would like to thank Dr Andrew Wragg for his help and advice with the writing of my thesis. I am also extremely grateful to Mr Rakesh Uppal who enabled the RIPC trial to be undertaken within the Department of Cardiothoracic Surgery. I would also like to thank Dr Matthew Lovell and Dr Dan Jones for teaching me what now appear to be relatively basic computing skills, and for their moral support over the last 3 years.

I am very grateful for the support of the Barts and the London Charities who funded the RIPC trial and made this thesis possible.

Declaration

I, Sean Gallagher, confirm that the work presented in this thesis is my own work, except where acknowledged in the text. This work is based on research that was undertaken by me at Queen Mary, University of London, during the period 6th October 2010 to 2nd October 2012.

In particular I developed and then analysed the cardiac surgical database. For the randomized control trial detailed in this thesis, I revised the study protocol, achieved Peer Review Committee and Research Ethics Committee approval. I recruited all of the study patients, collected all study samples, and analysed the eventual study data. I also performed all ELISA assays upon the urine and serum samples collected.

Any ideas or quotations from the work of other people are fully acknowledged in accordance with the standard referencing practices.

Sean Gallagher

17th June 2013

Publications arising from this research

Gallagher S, Jones DA, Lovell MJ, Hassan S, Wragg A, Kapur A, Uppal R and Yaqoob MM **The Impact of Acute Kidney Injury upon Mid-Term Outcomes Following CABG: A Matched Propensity Score Analysis** Accepted for publication Journal of Thoracic and Cardiovascular Surgery 2013

Gallagher S and Knight C **Contrast-induced nephropathy in primary percutaneous coronary intervention** Heart 2011;97(21):1723-5

Gallagher S, Kapur A, Lovell MJ, Jones DA, Kirkwood A, Hassan S, Archbold RA, Wragg A, Uppal R and Yaqoob MM **Impact of diabetes mellitus and renal insufficiency on five-year mortality following coronary artery bypass graft surgery: a cohort study of 4,869 UK patients** Accepted for publication European Journal of Cardiothoracic Surgery

Presentations to learned societies

Acute kidney Injury is Associated With Increased Mortality After Cardiac Surgery in Patients With Pre-Existing Renal Impairment. American Society of Nephrology, Philadelphia, 2011

Is Off-Pump Coronary Artery Bypass Grafting Preferable in Patients with Pre-Existing Renal Impairment? American Society of Nephrology , Philadelphia, 2011

The Impact of Acute Kidney Injury upon Long-Term Outcomes Following CABG: A Matched Propensity Score Analysis European Society Cardiology, Munich 2012

Estimated glomerular filtration rate and outcome following coronary artery bypass grafting: Impact on mortality after a 5.5-year follow-up European Society Cardiology, Munich 2012

The Effect of CKD and Diabetes Mellitus upon Outcomes after Coronary Revascularisation. Society for Cardiothoracic Surgeons of Great Britain, Manchester 2012

Chronic Kidney Disease and Diabetes Mellitus: Can We Do More to Modify Cardiovascular Risk After Coronary Artery Bypass Graft Surgery? American Society of Nephrology. Philadelphia, 2011

Remote ischaemic preconditioning does not prevent kidney injury after cardiac surgery in patients with chronic kidney disease British Renal Association Annual Conference, Bournemouth 2013

Chapter 1

1 Introduction

Each year in the United Kingdom approximately 40,000 cardiac surgical operations are performed [1]. The majority of these are isolated first time coronary artery bypass graft (CABG) operations which by cardiac surgical standards is a low-risk procedure; associated with an expected in-hospital mortality in most patients of less than 2%[2]. In recent years there has been an increase in the risk profile of patients undergoing cardiac surgery [1]. One patient group at particularly high risk of adverse events during cardiac operations are patients with pre-existing chronic kidney disease (CKD) [3]. A particular problem for these patients is the development of perioperative acute kidney injury (AKI). The development of AKI in patients undergoing cardiac surgical procedures is associated with a significant increased in both perioperative and long-term morbidity and mortality [4-6]. To date, attempts to modify outcomes after cardiac surgery in patients with CKD, in particular therapies to reduce the development of perioperative AKI, have been largely unsuccessful.

This thesis describes my research methodology and results obtained during my MD (Res) studies. It begins with a review of the cardiovascular complications of CKD. This naturally leads to a discussion of the management of cardiovascular disease in patients with CKD. I will also review the importance of AKI in cardiac surgery and highlight the current lack of effective strategies to prevent this important surgical complication.

1.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is the general term given to a heterogeneous group of disorders affecting the structure and/or function of the kidney. It is defined by the presence of albuminuria or reduced glomerular filtration rate [GFR] for 3 months or more. CKD is classified into five stages on the basis of GFR (**Table 1**) [7].

Table 1 Stages of chronic kidney disease (adapted from[7])

Stage	Description	eGFR ml/min
1*	Kidney damage with normal or increased GFR	≥90
2	Kidney damage with mildly decreased GFR	60-89
3	Moderately decreased GFR	30-59
4	Severely decreased GFR	15-29
5	Kidney Failure	<15 or RRT

End-stage renal disease (ESRD) is defined as the need for renal replacement therapy RRT (dialysis or renal transplantation).

*Stage 1 CKD is mostly recognized by either albuminuria or structural renal abnormality.

Further refinement of this classification system has been suggested by subdividing stage 3 CKD by GFR into stages 3A (45-59 mL/min) and 3B (30-44 mL/min) [8]. The wide range of GRF that defines CKD stage 3 means that patients with differing clinical patterns and risks are classified within the same stage. As many of the complications associated with CKD increase exponentially as GFR falls subdivision of CKD stage 3 allows differentiation of relatively low risk patients with CKD 3A from higher risk patients with CKD 3B.

In developed countries CKD is usually associated with ageing, diabetes, hypertension or vascular disease, with diabetic glomerulosclerosis and hypertensive nephrosclerosis the most frequently observed pathologies [9]. In developing countries CKD may result from glomerular or tubulointerstitial disease due to infection, drugs or toxins [9].

CKD is a current public health problem due largely to the increased risk of cardiovascular (CV) disease faced by these patients. CKD is estimated to affect 10% of adults in the western world [10, 11], with the worldwide prevalence of CKD increasing rapidly due to the 'diabetes epidemic', increased rates of hypertension and an ageing population [12].

1.1.1 CKD and Cardiovascular Risk

Patients with CKD are at particularly high risk of developing CV disease. This risk is highest in patients with end stage renal disease (ESRD), of whom approximately 50% will die of a CV cause [13]. It is now appreciated that the risk of developing CV disease is also

significant in patients with lesser degrees of renal dysfunction. For example, Go *et al* [14] recently described the relationship between GFR and CV disease in 1.1 million American adults that they followed for an average of 2.8 years. These investigators found that CV events (a composite of myocardial infarction, stroke, heart failure, and complications of peripheral vascular disease) increased markedly once GFR fell below 60 mL/min. Even after adjusting for potential confounders, GFR remained an independent predictor of CV events (**Figure 1**).

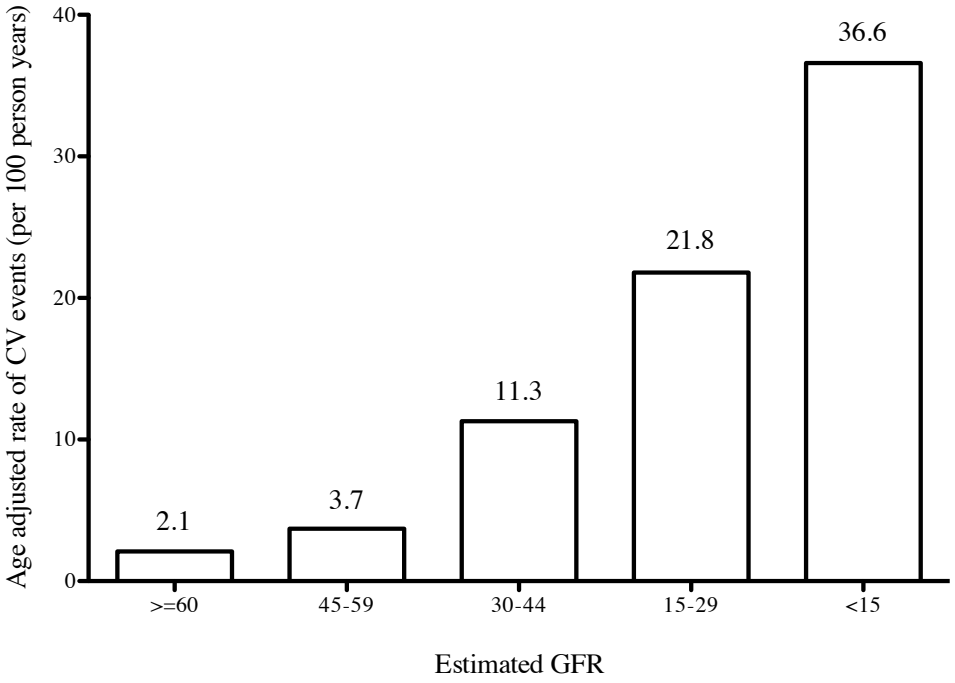


Figure 1 Age-adjusted rates of CV events by levels of eGFR(Adapted from [14])

This graded, inverse correlation between GFR and CV events is consistent in the literature, and appears to hold across populations with varying baseline CV risk. In the Hypertension Optimal Treatment (HOT) study, that enrolled more than 15000 hypertensive adult patients, the adjusted relative risks for all-cause mortality and for major CV events were 1.65 and 1.58, respectively, in subjects with an estimated glomerular filtration rate (eGFR) <60 mL/min when compared with those with an eGFR >60 mL/min [15]. In the Heart Outcomes Prevention Evaluation (HOPE) trial that involved more than 9000 patients with high baseline CV risk, an entry serum creatinine (sCr) of 124 mmol/L or higher was associated with a 40% increase in the risk of CV events during follow up {[16]. In a recent meta-analysis of the

relationship between GFR and CV risk, it was estimated that lowering GFR by 30% was associated with a 20%–30% higher risk of major CV events and all-cause mortality [17].

1.1.2 Albuminuria and Cardiovascular risk

Pathological albuminuria often precedes functional renal deterioration. Albuminuria is also recognized an important marker of CV risk. The association between albuminuria and CV events was first recognized in patients with overt macroalbuminuria (urine albumin:creatinine ratio [ACR] >300 mg/g) [18, 19], but this relationship has now been extended to more modest elevations of urinary albumin (ACR 30 to 300 mg/g) [20]. Recently it has been recognized that CV risk may rise within currently defined normal levels of albuminuria (ACR<30 mg/g). Thus, urinary albumin is a continuous CV risk factor, whereas microalbuminuria is a designated threshold for functional renal disease [21].

1.1.3 Arterial disease in patients with CKD

Arterial disease, particularly coronary artery disease (CAD) is especially common in patients with CKD. It results from two distinct arterial pathologies, namely atherosclerosis and arteriosclerosis [22]. Atherosclerosis is primarily an intimal disease, characterized by intima–media thickening and the formation of fibroatheromatous plaques. In patients with CKD there is marked plaque calcification and increased intima-media thickening. Arteriosclerosis is the thickening and calcification of the arterial media. It results in a reduction in arterial dampening (responsible for transforming pulsatile central arterial flow into steady peripheral flow) and an increase in systolic and pulse pressure. Arteriosclerosis causes an increase in left ventricular afterload and eventually the development of left ventricular hypertrophy (LVH), with a resultant increase in microvascular perfusion demand. Due to a reduction in myocardial capillary density and endothelial dysfunction which also occur in patients with CKD, the ability to match microvascular perfusion to increased myocardial oxygen demand is impaired, thus creating a state of chronic myocardial ischaemia that will result in myocardial fibrosis [23].

1.1.4 Causes of arterial disease in CKD

There is a clustering of traditional CV risk factors such as hypertension and diabetes mellitus in patients with CKD. However, these factors perform very poorly in the prediction of CV risk in this population, particularly in later stage CKD and these factors alone do not fully

explain the excess in arterial disease observed [24]. Novel 'uraemia' related factors are increasingly suggested as an explanation of the excess of arterial disease observed in these patients [24].

An understanding of the mechanisms underlying arterial disease in CKD is essential for devising strategies to prevent this pathophysiology. Below I will review the potential mechanisms that explain the increase in arterial disease observed in patients with CKD.

1.1.4.1 Hypertension

Hypertension is almost invariably present in patients with CKD. Sodium retention together with the aberrant hyperactivation of the renin-angiotensin-axis (RAA) and sympathetic nervous system are important mechanisms that contribute to hypertension in patients with CKD [25]. Also production of renalase in the glomeruli and proximal tubules of the kidney (an enzyme that breaks down catecholamines and is a potent hypotensive agent) is reduced in CKD [26]. Hypertension has direct deleterious CV effects such as LVH induction [23]. Indirectly, hyperactivation of the RAA causes oxidative stress, endothelial dysfunction and increased uptake of oxidised low-density lipoproteins by endothelial cells, accelerating atherosclerosis [27].

1.1.4.2 Dyslipidaemia

Dyslipidaemia affects approximately two-thirds of patients with CKD [28]. In CKD, hepatic synthesis of apolipoprotein-A1 (the major component of plasma HDL) is reduced, and plasma HDL levels fall. HDL is an important antioxidant protecting the endothelium from the effects of proinflammatory cytokines. Also in CKD, levels of apolipoprotein-CIII, a competitive inhibitor of lipoprotein lipase, are increased, leading to reduced hepatic uptake of triglycerides and a resultant increase in serum triglycerides. Reduced lipoprotein lipase activity also results in the accumulation of intermediate-density lipoproteins (which comprise very low-density lipoprotein and chylomicron remnants). Both serum triglyceride and intermediate density lipoproteins impair endothelial function and are associated with the development of CAD [29].

1.1.4.3 Inflammation

Atherosclerosis is thought to be an inflammatory condition. Atherosclerotic lesions represent a protective, inflammatory-fibroproliferative response to endothelial injury. In CKD there is chronic low-grade inflammation of the vascular wall that results in excessive inflammation and fibrous connective tissue proliferation that result in accelerated atherosclerosis [30]. Furthermore, in CKD chronic inflammation is linked to excessive vascular calcification [31]. Inflammation and atherosclerosis in CKD is complex, driven in part by traditional CV risk factors such as diabetes (due to the accumulation of advanced glycation end products in the vascular wall), hypertension (due to aberrant activation of RAA) and the modified lipoproteins associated with CKD dyslipidaemia, but also by an increase in oxidative stress, concurrent or chronic infection, or haemodialysis-related factors such as dialysis membrane biocompatibility [29].

1.1.4.4 ***Endothelial dysfunction and oxidative stress***

Endothelial dysfunction is a central pathophysiological mechanism in the development of atherosclerosis. It is thought to result from a reduction in nitric oxide (NO) bioavailability and an increase in oxygen free radical formation [32].

In patients with renal disease, endothelial dysfunction is ubiquitous. This is because uraemia is characterized by a reduction in the bioavailability of NO, predominantly due to an increase in vascular wall oxidative stress, but also in part due to a reduction in NO production as NO synthase is inhibited by asymmetric dimethyl arginine. In uraemia enhanced oxidative stress results from an increase in the generation of reactive oxygen species (ROS) by endothelial cells under the influence of nicotinamide adenine dinucleotide NAD(P)H oxidase (itself stimulated by the aberrant production of angiotensin-II) [33], myeloperioxidase (MPO), uncoupled endothelial NO synthase [34] and there is also reduced inactivation of ROS by antioxidant systems such as superoxide dismutase [35].

Asymmetric dimethyl arginine (ADMA) is a potent endogenous competitive inhibitor of NO synthase that contributes to the reduced availability of endothelial NO, and thus endotheilal dysfunction. In CKD, ADMA levels are increased; potential explanations for the increased levels of AMDA include accelerated ADMA production, decreased breakdown or reduced renal clearance [29]. Elevated plasma levels of ADMA have been associated with progression of renal disease and CV mortality in CKD populations [36].

1.1.4.5 Vascular calcification in renal disease

In CKD hyperphosphataemia develops as the kidneys fail to excrete excess dietary phosphate. Hyperphosphataemia suppresses the renal hydroxylation of inactive 25-hydroxyvitamin D to calcitriol eventually leading to hypocalcaemia. Thereafter, parathyroid hormone (stimulated by both hyperphosphataemia and hypocalcaemia) causes the mobilization of calcium from bone. Uraemia along with excessively elevated extracellular phosphate levels and possibly calcium levels are sufficient stimulus to cause the transformation of vascular smooth muscle cells into osteoblast-like cells that allow subsequent vascular calcification. Progressive mineralization then depends upon the balance of pro-calcific factors such as the calcium-phosphorus product, parathyroid hormone, and bone morphogenetic protein-2 and inhibitory factors such as the protein fetuin-A, pyrophosphate, osteopontin, osteoprotegerin, and γ-carboxyglutamic acid protein [29].

Patients with CKD have calcified atherosclerotic plaque disease in the intima, along with calcification of the media and premature calcific valvular heart disease [37]. Coronary artery calcification can be demonstrated in approximately 40% of patients with stage 4 CKD [38]. Coronary artery calcification, quantified by electron-beam computed tomography is associated with subsequent myocardial infacrtion (MI) [39].

1.1.4.6 ***Anaemia***

Anaemia is common in the CKD population, and its prevalence increases as GFR falls [40]. It is a marker of increased CV risk independent of GFR [41].

In CKD anaemia may be due to the combination of chronic disease, iron deficiency but mostly it is a result of a relative deficiency of erythropoietin (EPO) [42]. Anaemia causes and/or exacerbates LVH in patients with CKD, and EPO deficiency may result in a reduction in the production of bone marrow-derived endothelial progenitor cells, which may be associated with impaired endothelial repair mechanisms [43].

1.1.4.7 Hyperhomocysteinaemia

Homocysteine, a product of methionine metabolism, is predominantly excreted by the kidneys. Thus in renal failure homocysteine levels are raised [27]. Elevated homocysteine levels predict CV events in the general population and in patients with CKD [44]. In one

study, elevated homocysteine and fibrinogen levels were associated with an almost 40% increase in CV mortality in patients with CKD [45]. Trials of lowering homocysteine with folic acid and/or B vitamins have been largely unsuccessful at reducing CV events in the general population and in patients with CKD [44].

1.1.5 Diabetes and CKD

Currently in the UK, diabetes mellitus (DM) affects 2.8 million people (4.3% of UK population) [46]. Approximately 40% of adults with DM also have CKD characterized by either albuminuria or decreased GFR [47]. With population ageing and increasing obesity the prevalence of DM is predicted to rise by 20% by 2030 [48]. Consequently there will be a vast increase in patients affected by both DM and CKD in coming years.

DM is also associated with the premature development of CAD. When both CKD and diabetes are concurrent, the prevalence of CAD increases dramatically and risks of cardiac morbidity and mortality are particularly elevated. In an analysis of more than 1 million US patients both DM (RR 1.2) and non-diabetic CKD (RR 2.4) were found to be associated with an increase in CV mortality. Patients with the combination of DM and CKD had the highest risk of subsequent CV mortality (RR 2.6). In addition, in this analysis atherosclerotic vascular disease was evident in 35.7% of patients with non-diabetic CKD but evident in 49.1% patients with both DM and CKD [49].

The particularly high prevalence of CAD, and subsequent CV risk found in patients with both DM and CKD may be explained by atherogenic mechanisms common to both conditions such as dyslipidaemia, endothelial dysfunction, oxidative stress, systemic inflammation and homocysteinaemia [50]. It is probable that these mechanisms are augmented in patients with both DM and CKD.

In addition to these ‘shared’ atherogenic mechanisms, hyperglycaemia and insulin resistance that define DM can further disrupt normal endothelial function and enhance atherosclerosis.

1.1.5.1 Hyperglycaemia and insulin resistance

Hyperglycaemia inhibits the NO production by blocking eNOS synthase activity [51]. It also activates the intracellular second messenger protein kinase-C (PKC) [51], producing ROS which react with NO to produce peroxinitrate [52]; a cause of direct endothelial damage.

Insulin-resistance is associated with less NO production and more vasoconstrictor production, most notably endothelin-1 (ET-1) [53]. Also insulin-resistance causes liberation of free fatty acids from adipose tissues, activating PKC, inhibiting phosphatidylinositol-3 (PI-3) kinase (an eNOS agonist pathway) and thus increasing ROS production [53]. Hence the combination of hyperglycaemia and insulin-resistance is associated with reduced bioavailability of NO and increased vasoconstrictor production, which are central mechanisms in endothelial dysfunction.

1.1.5.2 Advanced Glycation Endproducts (AGEs)

Both hyperglycaemia and oxidative stress cause the generation of AGEs by non-enzymatic glycation of proteins and lipids in the arterial wall [54]. In both DM and CKD AGEs concentration in plasma and AGE formation in vascular tissues is increased [55]. The kidney usually excretes AGEs and so they accumulate in renal failure [55]. Thus in patients with DM and CKD the generation of AGEs are enhanced, but excretion reduced.

AGEs affect the vasculature both directly and indirectly via cell surface receptors for AGEs (RAGE) [54]. AGEs are found in accelerated atherosclerotic lesions, where they may induce collagen crosslinking, resulting in thickening and stiffening of arterial walls [55]. AGEs also inhibit the bioavailability and activity of endothelium-derived NO and so directly contribute to endothelial dysfunction. Binding of AGEs to cell surface RAGE induces intracellular ROS production along with expression of proinflammatory cytokines and adhesion molecules associated with inflammation. Thus AGEs potentiate the development of atherosclerosis [54].

1.1.5.3 Impaired platelet function

Hyperglycaemia affects platelets in the same fashion that it affects the endothelium; reducing NO production, activating PKC and increasing oxidative stress [53]. DM also affects platelet

calcium homeostasis, which is vital for regulating platelet shape change, platelet aggregation and thromboxane production [53]. Finally, hyperglycaemia increases platelet surface expression of glycoprotein 1b (Gp1b). Gp1b mediates the platelet fibrin interaction in thrombus formation [53]. Platelets are modulators of vascular function, thus these abnormalities of platelet function may potentiate atherosclerosis progression.

1.1.5.4 Abnormal Coagulation

DM can be considered a procoagulant state. Coagulation factors such as plasminogen activator inhibitor-1 (PAI-1), von willebrand Factor (vWF), fibrinogen, factor VII and thrombin–antithrombin complexes are increased resulting in a propensity for coagulation [56]. Fibrinolytic pathways are also suppressed in patients with DM in association with insulin resistance [53]. The procoagulant state of DM may predispose the thrombotic complications of CAD.

1.1.6 Management of coronary artery disease in CKD

Managing CAD is particularly challenging in patients with CKD. Firstly clinical diagnosis is difficult as patient symptoms are often atypical or absent. Furthermore many investigations for CAD are less useful in patients with CKD than in the general population. For example, due to the high prevalence of resting ECG abnormalities and a reduced exercise capacity in many patients with CKD the exercise ECG is of limited diagnostic value with a reported sensitivity as low as 30% [57]. As such most patients with CKD require pharmacological stress imaging with either adenosine or dobutamine for diagnosis. Nuclear myocardial perfusion imaging (MPI), although more sensitive than the exercise ECG, is limited by LVH and endothelial dysfunction, which may cause false positive perfusion defects [58] and thus the specificity of nuclear MPI is approximately 70% in this population [57]. Dobutamine stress echocardiography (DSE) can be both sensitive and specific, but is highly operator dependent. The recent association between gadolinium based contrast agents and the development of nephrogenic systemic fibrosis (NSF) in patients with low GFR has limited the application of adenosine stress cardiovascular magnetic resonance (CMR) in patients with CKD. Computed tomographic coronary angiography (CTCA) is a non-invasive anatomical imaging alternative to invasive coronary angiography. Unfortunately, calcific plaque disease limits the diagnostic accuracy of CTCA in patients with CKD. Furthermore, the risk of contrast induced nephropathy (CIN) means CTCA is rarely used in this patient

population. Thus, invasive coronary angiography remains the gold standard for the diagnosis of CAD and is a prerequisite for any revascularisation strategy.

1.1.6.1 Treatment of CAD in CKD

1.1.6.1.1 Pharmacological Therapy

Pharmacological therapy is the mainstay of treatment for CAD, and is underused in patients with CKD compared with the general population. This is most probably due to concerns about limited efficacy, potential toxic effects and ignorance of the potential benefits of pharmacological therapy in this population (a concept termed 'therapeutic nihilism') [59].

Pharmacology for the primary prevention of CAD in CKD: In view of the high prevalence of CAD in patients with CKD, a strategy of primary prevention with aggressive targeting of both traditional and uraemia related risk factors is advocated in guidelines published by the UK based Renal Association and the US National Kidney Foundation (**Table 2**).

Pharmacology for the secondary prevention of CAD in CKD: Anti-platelets, beta-blockers, angiotensin converting-enzyme (ACE) inhibitors and statins are established effective treatments proven to reduce CV morbidity and mortality in patients with arterial disease. However, most major clinical trials evaluating these therapies have systematically excluded patients with renal insufficiency, and therefore the treatment of CAD in patients with CKD is often not evidence-based.

Antiplatelet therapy: Aspirin is underused following acute myocardial infarction (AMI) in patients with CKD. In the US National Cardiovascular Data ACTION (Acute Coronary Treatment and Intervention Outcomes Network) registry, including nearly 50,000 patients with AMI, aspirin prescription decreased as severity of renal disease increased [61]. The efficacy of aspirin for secondary prevention after an AMI is undiminished in CKD. Aspirin is most likely underused due to safety concerns, in particular bleeding risk. Uraemia is associated with platelet dysfunction and prolonged bleeding times. However, in the UK HARP (Heart and Renal Protection)-1 trial chronic low-dose aspirin (100 mg/day) use was not associated with an increase in major bleeding or worsening of renal function in patients

with CKD [62]. There are no controlled data on the efficacy of long-term aspirin use in CKD patients diagnosed with stable coronary artery disease.

International guidelines recommend combining aspirin and clopidogrel post-AMI. There are no specific recommendations for adjustment of clopidogrel dosing in patients with renal disease. Clopidogrel appears to be clinically efficacious in patients with lower GFR, although there is more minor bleeding (but not major bleeding) in patients with impaired renal function who are prescribed clopidogrel post AMI [63, 64].

Table 2 Treatment recommendations for cardiovascular risk factors in patients with CKD (Adapted from [57])

	Recommendation
Smoking	Stop
Obesity	Lose weight to improve insulin resistance, hypertension, dyslipidaemia
Hypertension	<140/<90 mmHg for all patients with CKD. For patients with a urinary protein/creatinine ratio > 100 mg/mmol and diabetics with microalbuminuria BP shpuld ideally be <130/<80. Initial treatment should be an ACE inhibitor or ARB [60]. If creatinine rises >20% or GFR falls >15%, repeat tests and consider referral for exclusion of renal artery stenosis. Do not stop treatment for a lesser rise in creatinine or GFR change.
Dyslipidaemia	Total cholesterol < 4mmol/L (or 25% reduction from baseline); LDL <2.0 mmol/L (or 30% reduction from baseline). Statins should be initial therapy.
Diabetes	HbA1c <7.5%
Albuminuria	Reduce as much as possible: Angiotensin II inhibition treatment of choice.
Antiplatelets	Indicated for secondary prevention as no evidence for beneficial effect in primary prevention of CVD
Calcium/phosphate product	Maintain calcium and phosphate levels in normal range; PTH in patients on RRT 2–9 x upper limit of normal

Lipid lowering therapy: Statins are used for the primary and secondary prevention of atherosclerosis in the general population. Their role in the primary prevention of atherosclerosis in CKD patients remains to be clarified. Despite reductions in low-density lipoprotein and a significant decrease in CV events, no significant reduction in mortality has

been shown with statin therapy in patients with CKD [65]. This may be because most deaths in patients with CKD are due to cardiac failure or arrhythmia rather than acute coronary events [47]. For secondary prevention statins are safe and effective in patients with mild CKD [66, 67], although evidence of benefit of statin therapy in patients with more advanced renal disease is limited. There is evidence that statin use may slow the rate of decline of renal function in patients with CKD through anti-inflammatory effects on the glomerulus and tubules meaning that statins may be beneficial in CKD in a manner independent of lipid lowering [57].

Hypertension management: Target blood pressure in patients with CKD is <130/80 mm Hg, or <125/75 mm Hg for patients with significant proteinuria > 1 g/day [60]. In CKD, hypertension is volume-dependent. Hence maintenance of fluid balance with careful oral restriction of fluid intake and judicious diuretic use is paramount.

ACE inhibitors form the mainstay of antihypertensive therapy in most patients with CKD and slow the decline in renal function. Their 'renoprotective' effect is due in part to their antihypertensive effect, but also a specific antiproteinuric effect [57]. As CV mortality is intimately related to renal function, it seems logical that the renoprotective effects of ACE inhibition would lead to improved CV outcomes. In the HOPE trial, ACE inhibitors reduced CV mortality more so in patients with CKD than in those with normal renal function [16]. Angiotensin receptor blockers (ARBs) are also renoprotective. However, ARBs have not been shown to reduce CV events in patients with CKD. Due to the lack of proven cardioprotective effect of ARBs in CKD, ACE inhibitors are preferred when possible.

β-blockers can be particularly useful in CKD, both as an antihypertensive agent and an anti-anginals. However, due to the high prevalence of sudden cardiac death in patients with ESRD perhaps the most important role of β-blockers is as an anti-arrhythmic agent. In a study of haemodialysis patients with LV dysfunction, those treated with β-blockers showed an improvement in LVEF of almost 10% over 12 months and a 49% reduction in all cause mortality at 2 years, compared with similar patients not receiving β-blockers [68]. This beneficial effect of β-blockade is not restricted to patients with severe renal dysfunction; in an analysis of over 200,000 patients with mild CKD, β-blocker therapy was associated with a 35% reduction in mortality [69].

Management of anaemia: Anaemia is a strong CV risk factor in CKD. It may cause angina, decreased exercise tolerance and induce LVH. It can be treated with erythropoietin-stimulating agents (ESA) although, two large studies investigating the effect of haemoglobin correction with ESA (to levels >13 g/dL) in patients with advanced renal disease did not show any improvement in CV outcomes compared to lower haemoglobin levels [70, 71]. Also the risk of stroke may be increased with overzealous haemoglobin correction with ESA [72]. As a result target haemoglobin level of between 10–11.5 g/dL would be considered optimal for most patients with CKD [73], however any therapy for anaemia (ESA or blood transfusions) should only be administered after carefully weighing the risks of the therapy (ie. stroke or vascular complication), against the benefits of relieving symptoms secondary to anaemia.

Calcium/phosphate product: Elevated calcium/phosphate product and parathyroid hormone (PTH) are associated with an increased incidence of CV disease and increased mortality in patients with CKD [74]. No randomised trials have proven that treating secondary hyperparathyroidism and hyperphosphataemia alters CV outcome. Guidelines suggest that calcium and phosphate levels are maintained within normal range for patients with advanced CKD [75].

Hyperglycaemia: The relationship between glycaemic control and the microvascular complications of DM is well established. Tight glycaemic control has consistently been shown to prevent the onset of microalbuminuria [76]. Once CKD is established it is unknown whether tight glycaemic control retards the progression of renal disease.

The effect of tight glycaemic control upon CV complications in patients with T2DM is questionable. For patients with T1DM targeting an HbA1C level of 6%, reduced CV outcome by 42% over 17 years of follow up when compared with conventional DM therapy [77]. In T2DM, trials targeting HbA1C levels of < 6–6.5%, despite confirming a reduction in microvascular complications, have not shown a reduction in the incidence of CV events [78, 79]. In fact in the ACCORD trial the overall and CV mortality was higher in patients with T2DM treated with intensive glycaemic therapy [79].

Published guidelines recommend HbA1c levels <7.0% for all stages of renal function, but that lower HbA1C levels may be appropriate in young patients with little comorbidity,

whereas higher HbA1C levels may be accepted in patients at high risk hypoglycaemic events [75].

1.1.6.1.2 Coronary revascularization

Coronary revascularization, either with percutaneous coronary intervention (PCI) or CABG is indicated for the relief of angina, improvement of prognosis, or both. It may be performed in patients with stable coronary disease or following an acute coronary syndrome.

Stable coronary disease: In patients with stable CAD, PCI is a symptomatic treatment. It provides superior angina relief to optimal medical therapy (OMT) but does not reduce risk of subsequent myocardial infarction (MI) or death [80]. Whether PCI of a coronary stenosis causing a large amount of ischaemic myocardium has a beneficial prognostic effect is unknown. Whether PCI of a coronary stenosis causing a large amount of ischaemic myocardium has a beneficial prognostic effect is currently unknown. However, there is limited data to suggest that PCI of a coronary stenosis causing ischaemic myocardium may be associated with improved prognosis [81, 82].

CABG is a highly successful surgical treatment indicated for the relief of angina not controlled by OMT. It has also been associated with improved survival in selected patients with a greater than 50% stenosis of the left main coronary artery [83], or three-vessel coronary artery disease with left ventricular dysfunction [84].

When extrapolating these indications for revascularisation to the CKD population it is important to consider,

1. the application rate of OMT in patients with CKD
2. the efficacy of revascularization in CKD
3. the risks of revascularization in CKD

Percutaneous coronary intervention: Prior to the advent of intracoronary stents, balloon only angioplasty was associated with unacceptably high rates of procedural complications (~10%) and restenosis (~80%) in patients with CKD [85]. However since the advent of coronary stents early success rates of PCI are now similar among patients with CKD and those without. Restenosis following PCI remains a problem in patients with CKD, although drug-eluting stents (DES) have been shown to reduce in-stent restenosis (ISR) compared

with bare metal stents (BMS) in both the general population and in patients with CKD; for example an analysis of the TAXUS-IV database showed that the implantation of paclitaxel-eluting stents was associated with lower 9-month re-stenosis rates (2.1% vs. 20.5%, $p < 0.01$) and one-year target vessel revascularisation (TVR) rates (3.3% vs. 12.2%, $p < 0.001$) than BMS in patients with CKD [86].

Table 3 Studies examining morbidity and mortality in patients with renal disease following PCI

Year (Ref)	N	Study Groups eGFR cutoff: number of patients	Early Mortality Time point: (%)	Late Mortality Time point: (%)	Early MACE Time point: (%)	Late MACE Time point: (%)
2002	5327		**In-hosp**	**1-yr**		**1 yr**
[87]		GFR ≥70: BMS 2687	0.5	1.5		23.2
		GFR 50-69: BMS 1537	0.7	3.6		26.2
		GFR 30-49: BMS 899	2.3	7.8		30.4
		GFR <30: BMS 154	7.1	18.3		38.1
		ESRD: BMS 50	6.0	19.9		38.5
2004	11187		**30-day**	**9-mon**	**30-day**	**9-mon**
[88]		GFR >89: BMS 5384	0.07	0.8	1.17	15.4
		GFR 60-89: BMS 4054	0.27	1.16	1.23	14.5
		GFR <60: BMS 1749	0.17	2.16	1.66	16.6
2006	6840		**In-hos**		**In-hos**	
[89]		GFR >80: BMS 3474	0.3		2.4	
		GFR 61-80: BMS1670	0.7		3.0	
		GFR 41-60: BMS1111	1.5		4.8	
		GFR ≤ 40: BMS 585	6.0		9.7	
2006	371		**30-day**	**9-yrs**		**9 yr**
[90]		GFR≥60: BMS 269	0.75	7.1		39
		GFR<60, no HD: BMS 102	9.8	35.4		57
2007	2758		**In-hosp**	**2-yrs**		**2-yrs**
[91]		GFR ≥ 60 DES: 2093	0.5	4.7		15.3
		GFR <60 DES: 665	5.3	17.7		25.6
2005	1314			**1-yr**		**1-yr**
[92]		GFR >90 BMS: 346		1.2		22.3
		GFR >90 SES: 312		1.3		9.7
		GFR 60-89 BMS: 200		0.6		17.1
		GFR 60-89 SES: 219		0.9		10.7
		GFR <60 BMS: 100		3.0		19.1
		GFR <60 SES: 123		1.6		13.1
2006	1012	CKD=GFR<60	**In-hosp**			**17-mon**
[93]		No CKD: 602	1			13.8
		CKD: 410	3.4			18.3
		CKD+ DES: 264	3			15.2
		CKD+ BMS: 146	4.3			24.7
2007	304			**8-mon**		**8-mon**
[94]		GFR ≥ 60 SES: 204		0		10.8
		GFR <60 SES: 69		2.9		18.8
		HD SES: 31		3.2		38.7
2009	4791		**In-Hosp**	**1-yr**		**1 yr**
[95]		GFR > 75: 2827	0.1	1.5		18.0
		GFR 50-75: 1253	0.2	3.5		22.3
		GFR 30-49: 571	0.9	5.8		21.5
		GFR <30: 140	0.0	13.6		

Both early and late morbidity and mortality are consistently higher in patients with CKD than the general population following PCI (**Table 3**). In summary, despite encouraging procedural success rates, and reduced restenosis rates with DES, CKD remains a strong independent predictor of adverse outcomes following PCI.

Coronary artery bypass graft surgery: There are an abundance of studies reporting outcome after coronary surgery in patients with non-dialysis dependent CKD, but most have categorised patients using arbitrary serum creatinine (sCr) values to define renal dysfunction rather than the more discriminating eGFR. This causes problems in comparing outcomes between surgical series, but also using these creatinine cutoffs to define CKD may underestimate its prevalence within a given population.

In general CABG surgery is associated with higher levels of early post-operative complications and early mortality in patients with CKD than the general population. For example, in an analysis of the US National Adult Cardiac Database including approximately half a million patients stratified by eGFR (mild CKD, eGFR 60–90 mL/min; moderate CKD, eGFR 30–60 mL/min; severe CKD, eGFR <30 mL/min and those requiring dialysis), CKD was prevalent (mild CKD 51%, moderate CKD 24%, severe CKD 2% and ESRD 1.5%) and a powerful independent predictor of operative mortality and early complication including stroke, prolonged ventilation, deep sternal wound infection, prolonged hospital stay and new dialysis requirement. Operative mortality was 1.3% in those with normal renal function but as high as 9% in those with severe CKD or ESRD [3]. Long-term mortality is also higher in patients with CKD after CABG than in patients with normal renal function. In a cohort study of 2,067 consecutive CABG operations, patients with moderate CKD (eGFR <60 mL/min) were twice as likely to have died at median follow-up of 2.3-years than patients with an eGFR >60mL/min. Although patients with CKD had an excess of co-morbidity, after adjusting for this, CKD remained a strong independent predictor of long-term mortality [96].

However despite the increase in early and late mortality following CABG observed in patients with CKD it is important to remember that the prognostic advantage of CABG compared to medical therapy in selected patients with multivessel CAD is unabated despite the coexistence of CKD [97]. Thus the increased risks associated with coronary surgery should not act as a deterrent to surgical coronary revascularization in patients with CKD. The challenge for cardiac surgeons, cardiologists and nephrologists is to develop new therapies and techniques to make coronary surgery safer within this high-risk population. As

such this thesis focuses upon developing novel strategies to make cardiac surgery safer for patients with CKD.

Summary

CKD is an increasing world health problem, associated with increased CV morbidity and mortality. The mechanisms underlying this increased CV risk are diverse and far more complicated than in the general population. CAD in patients with CKD is uniquely different from CAD in patients with normal renal function, with increased vascular stiffness, more calcification and a particularly aggressive disease course. Consequently, treatment of CAD in patients with CKD is challenging, especially coronary revascularization. Both PCI and CABG are associated with higher rates of adverse events than are seen in patients with normal renal function. Novel strategies to make coronary revascularization safer for patients with CKD are now urgently needed.

1.2 Acute kidney injury following cardiac surgery

Acute kidney injury (AKI) is a significant problem following cardiac surgery and is especially common in patients with CKD. As AKI after cardiac surgery is a central concept within this thesis it is therefore reviewed in detail below;

The incidence of AKI following cardiac surgery varies from 1% to 30% depending upon how it is defined and population that is studied [5, 6, 98-107]. The reported frequency of AKI requiring post-operative renal replacement therapy (RRT) is generally lower ranging from 1% to 6%[5, 6, 98-107]. AKI following cardiac surgery is a serious event, associated with an increase in post-operative infectious complications, increased length of hospital stay and an increase in post-operative mortality [4-6] which can exceed 60% among patients requiring post operative RRT [100]. Despite advances in our understanding of the aetiology and pathophysiology of AKI after cardiac surgery there has not been a significant reduction its incidence [108] nor the mortality associated with this complication [109].

1.2.1 Defining Cardiac Surgery Associated Acute Kidney Injury

Cardiac Surgery Associated Acute Kidney Injury (CSA-AKI) is an impairment of renal function, either of new onset or an exacerbation of pre-existing renal dysfunction that follows cardiac surgery. Until recently a standardized definition for CSA-AKI was lacking. This absence of consistency in the diagnosis of CSA-AKI made the comparison of results between studies into CSA-AKI extremely challenging. For example, CSA-AKI has been diagnosed by absolute or percentage changes in sCr value, or eGFR, by reduction in urine output post-operatively or the need for post-operative RRT [5, 6, 98-107]. In order to standardize the definition of CSA-AKI, the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) proposed a graded classification system for the diagnosis of CSA-AKI [110]. This classification system defined 3 grades of increasing severity of AKI (risk, injury, and failure) on the basis of changes of sCr or glomerular filtration from baseline as well as reductions in urine output, and has 2 final stages that are outcome variables (loss and end-stage kidney disease), thus the acronym of the RIFLE classification [110]. To further refine this grading system for CSA-AKI, the Acute Kidney Injury Network (AKIN) proposed a modification of the RIFLE classification, known as the AKIN classification [111]. The differences between RIFLE and AKIN staging criteria are subtle (**Table 4**).

Despite the advantages of using these standardized, graded definitions for CSA-AKI, both classification systems have limitations. sCr and urine output perform poorly as surrogates of AKI. sCr is influenced by many factors independent of the kidney, including hydration and nutritional status, gender, age, race, and muscle mass, and can vary by as much as 20% each day [108]. Furthermore, due to extensive intrinsic renal reserve, the glomerular filtration rate must decrease by approximately 50% before sCr changes significantly from baseline [108], and as such changes in sCr may lag by upto 48 to 72 hours behind the initial renal insult [112].

1.2.2 Prognostic Implications of CSA-AKI

The development of CSA-AKI is clearly associated with an adverse prognosis. Early mortality is highest in patients with CSA-AKI requiring post-operative hemodialysis, and may exceed 60% [100]. However, these patients often have multiple-organ failure, and so the direct contribution of AKI to mortality is difficult to discern. Importantly, smaller

reductions in renal function following surgery without obvious immediate clinical sequelae are also associated with increased early mortality [106, 113].

The prognostic implications of CSA-AKI are not confined to the short term. Hobson *et al* detailed the association between long-term mortality and CSA-AKI defined by the RIFLE criteria. These authors found that CSA-AKI was an important independent predictor of 10-year mortality. At this time point the mortality rate was 56% in the patients who had previously developed CSA-AKI compared with 37% in patients without CSA-AKI [6]. Further to this, recent studies have reported that both the duration of CSA-AKI [99] and recovery of renal function after CSA-AKI also predict long-term survival following cardiac surgery[114].

The crucial question as to whether CSA-AKI has a causal relationship with subsequent mortality, or whether the development of CSA-AKI is simply a reflection of comorbidity and procedural complexity within a high-risk population remains unresolved. Despite attempting to account for confounding factors, it remains a distinct possibility that CSA-AKI is associated with mortality as it occurs in patients with a worse profile of pre-operative comorbidity who would have a poor post-surgical prognosis independent of the development of CSA-AKI.

1.2.3 Financial Implications of CSA-AKI

Inevitably, hospital costs and length of stay for patients affected by CSA-AKI are higher than unaffected patients [5]. Patients with CSA-AKI have been shown to incur higher intensive care unit (ICU) costs (1.7-fold), pharmacy costs (2.3-fold), and laboratory costs (1.6-fold). Patients requiring postoperative RRT had a doubling of post-operative costs and a tripling of ICU costs [115].

Table 4 Classification systems for acute kidney

RIFLE criteria*[110]			AKIN criteria+[111]		
Stage	GFR or sCr	Urine output	Stage	sCr	Urine output
Risk	sCr increase x 1.5 or GFR decrease >25%	<0.5 mL/kg/h for > 6 hours	1	sCr increase x 1.5 or sCr increase >26.5 μmol/L from baseline	<0.5 mL/kg/h for > 6 hours
Injury	sCr increase x 2.0 or GFR decrease >50%	<0.5 mL/kg/h for > 12 hours	2	sCr increase x 2 from baseline	<0.5 mL/kg/h for > 12 hours
Failure	sCr increase x 3.0 or GFR decrease >75% or sCr >353.6 μmol/L with an acute rise >44.2 μmol/L	<0.3 mL/kg/h for > 24 hours; or anuria > 12 hours	3	sCr increase x 3 or sCr increase >353.6 μmol/L with an acute increase >44.2 μmol/L	<0.5 mL/kg/h for >24 hours; or anuria > 12 hours
Loss	Persistent acute renal failure (complete loss of kidney function) >4 weeks				
ESRF	End-stage renal failure >3 months				

* Renal assessment time window upto 7 days. + Renal assessment time window upto 48 hours.

1.2.4 Risk factors for CSA-AKI and pre-operative risk prediction models

The pre-operative estimation of risk of developing CSA-AKI is vital to individualize and optimize peri-operative care. At least six validated CSA-AKI risk-prediction models currently exist (**Table 5**). Most of these models predict the need for RRT after cardiac surgery. Only one model predicts less severe CSA-AKI, defined as an eGFR < 30mL/min post-operatively in patients with 'normal' pre-operative renal function (eGFR > 60mL/min) [98].

Most risk models use broadly similar categories of variables; including patient related factors, surgery specific factors, and peri-operative physiologic insults. Common risk factors to all these models include advanced age, diabetes mellitus, congestive heart failure, and pre-operative CKD. Of these, pre-operative CKD is by far the most important risk factor for CSA-AKI. The risk of requiring post-operative RRT approaches 20% in patients with a

baseline sCr of 176.8 to 353.6 mmol/L and is approximately 25% when the baseline sCr c is greater than 353.6 mmol/L [109]. Other established patient related CSA-AKI risk factors include female gender, and left ventricular ejection fraction lower than 40%. Surgery specific CSA-AKI risk factors include the need for emergency surgery, peri-operative use of inotropic/device support and increasing procedural complexity; valve procedures are associated with a higher risk compared with CABG alone; with the highest risk for CSA-AKI found after combined CABG-valve procedures [109].

To date no risk model exists that predicts CSA-AKI defined by either RIFLE or AKIN criteria in an all-comer population. The development and validation of a risk model of this type would not only be of benefit to patients undergoing cardiac surgery, but would also be a powerful research tool providing a means for pre-operative selection of patients for trials of reno-protective therapies.

Table 5 Risk prediction models for CSA-AKI

	First author reference					
	Mehta [104]	Chertow [100]	Thakar [106]	Wijeysundera [107]	Brown[98]	Palomba [105]
Risk prediction model for	Post-operative RRT	Post-operative RRT	Post-operative RRT	Post-operative RRT	CSA-AKI [a]	Post-operative RRT
Number of patients, n	449,524	43,642	33,217	20,131	8,363	603
Operation type, %						
CABG only	78.6	81.5	na	65	100	53
Valve ± CABG	20.4	18.5	na	35	0	47
Elective surgery, %	54	74.0	na	59	28	na
Post-operative RRT, n						
Overall, %	6451	460	269	139	31	11
CABG only, %	1.4	1.1	1.7	1.3	0.4	1.8
	1.1	0.9	na	na	0.4	na
Independent predictors for AKI	Age Pre-op sCr Diabetes Surgery type Shock Prior MI NYHA class Reoperation COPD Etnicity	EF<35% Pre-op sCr SBP Surgery type Preop IABP NYHA class Reoperation COPD PVD	EF<35% Pre-op sCr Diabetes Surgery type Preop IABP Emergency NYHA class Reoperation COPD Female	EF<40% eGFR Diabetes Surgery type Preop IABP Emergency NYHA class CVP >14 cm	Age Female Diabetes PVD Preop IABP Hypertension NYHA class Reoperation WCC>12,000	Age >65 Preop sCr Diabetes Surgery type CPB >120m LCO
AUROC	0.83	0.76	0.81	0.81	0.72	0.84

[a] CSA-AKI defined as post operative eGFR<30mls/min. LCO: low cardiac output

1.2.5 Pathology of CSA-AKI

The pathophysiological processes thought to explain the development of CSA-AKI are depicted in **Figure 2**. The proposed first phase of CSA-AKI is a vasomotor nephropathy, in which there is an absolute or relative reduction in renal perfusion (either global or regional), effecting a reduction in GFR and an increase in both sCr and blood urea; this is usually but not always accompanied by oliguria. Subsequent consequences of renal hypoperfusion are cellular adenosine triphosphate depletion, and oxidative injury. This leads to an activation of inflammatory cells, renal epithelial cells and endothelial cells (initiation phase) [116, 117]. Inflammatory cells adhere to an activated endothelium in the peritubular capillaries of the outer medulla, with medullary congestion and hypoxic proximal tubular injury (extension phase) [116, 117]. Tubular cells will then proliferate and redifferentiate (maintenance phase), followed ultimately by the reconstitution of polarity and function (repair phase) [116, 117].

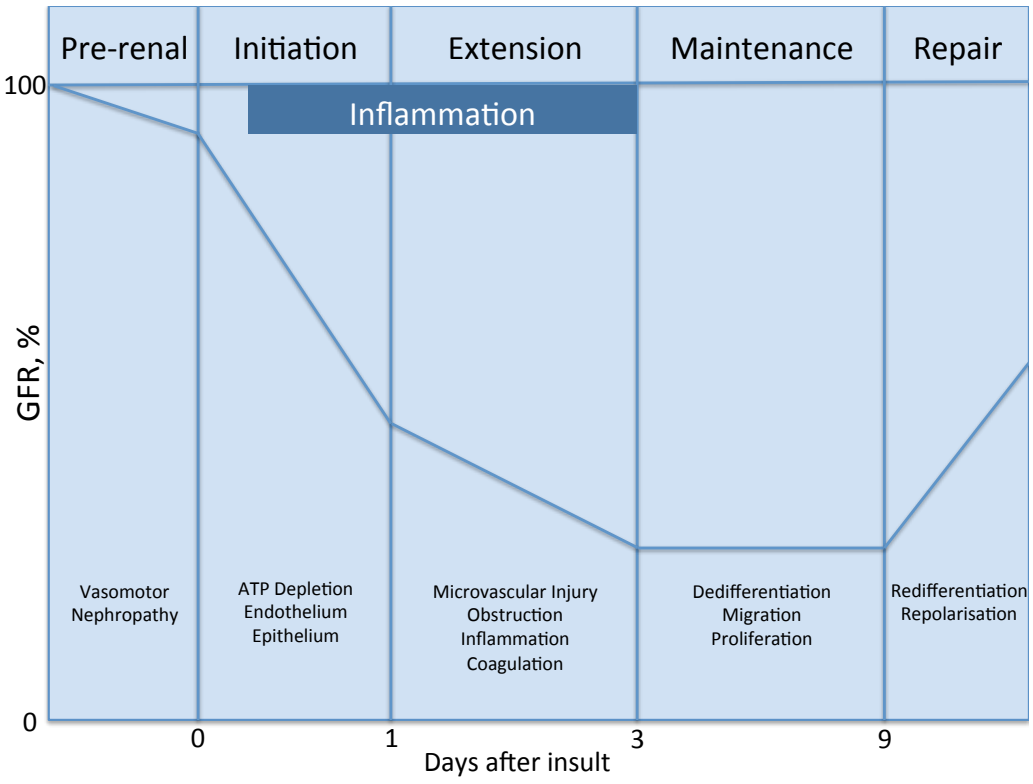


Figure 2 Conceptual model of CSA-AKI [117]

1.2.6 Pathogenesis of CSA-AKI

Aetiological factors central to the pathogenesis of CSA-AKI can be divided into preoperative, intraoperative, and postoperative events.

1.2.6.1 Preoperative events

Many patients undergoing cardiac surgery are pre-disposed to the development of CSA-AKI. Recent myocardial infarction, valvular heart disease and reduced left ventricular function may all cause a pre-operative reduction in renal perfusion. Furthermore, treatments for cardiac disease (such as diuretics) may impair the autoregulation of renal blood flow, and iodinated contrast used during coronary angiography may cause direct nephrotoxicity. Finally, a significant proportion of patients undergoing cardiac surgery have pre-existing CKD and thus lack 'renal-reserve', compounding the risk of CSA-AKI.

1.2.6.2 Intra-operative events

The intraoperative period is a crucial time when patients are exposed to anesthesia and cardiopulmonary bypass (CPB). Reduced renal perfusion pressure, activation of a systemic inflammatory response, and direct effects of the CPB circuit may all contribute to the development of CSA-AKI.

The goal of CPB is to maintain tissue perfusion at a level that supports optimal cellular function. Any reduction in tissue perfusion during CPB can lead to ischaemic cellular injury. The kidneys are particularly prone to ischemic injury as the renal medulla is normally perfused at a low oxygen tension with a limited reserve [118]. Maintenance of stable tissue perfusion during CPB requires interplay between blood flow generated by the CPB pump, systemic vascular resistance, and the autoregulatory capacity of end vascular beds. Standard haemodynamic goals of CPB are a mean perfusion pressure of 50 to 70 mmHg and CPB blood flow rates of 1.8 to 2.4 L/min per m^2. These haemodynamic parameters maintain cerebral blood flow autoregulation during CPB [119]; however the effect of these parameters upon regional renal blood flow is largely unknown. It is likely that renal perfusion and autoregulation are maintained as long as these parameters are met [120], but these values likely represent the minimum blood flows that support normal cellular function, and any further perturbation may lead to an oxygen supply/demand imbalance, with resultant renal ischaemia/reperfusion injury. Furthermore, these 'standard parameters' may be inadequate to

achieve adequate tissue perfusion in some patients undergoing CPB. Hypertensive patients and patients with diabetes are thought to have a 'right-shifted' tissue autoregulation curve [121], and so may require higher perfusion pressures to achieve optimal tissue perfusion. In addition, preexisting ATN or CKD may also impair the autoregulatory capacity of the kidney [122] and so renal blood flow becomes linearly dependent on pressure, increasing the likelihood of an already 'vulnerable' kidney sustaining a further ischaemic insult.

Other CPB related factors that may be implicated in CSA-AKI include haemodilution, hypothermia and the absence of pulsatile blood flow during CPB [118]. Haemodilution is necessary during CPB to reduce blood viscosity during hypothermia, but results in reduced tissue oxygen delivery. Hypothermia during CPB reduces metabolic demand [118], but nephron damage may occur due to hypoperfusion of the superficial cortex during rewarming [123].

The CPB circuit is a potent pro-inflammatory stimulus that can provoke a systemic inflammatory response syndrome (SIRS) [109, 118]. Contact of blood components with the artificial surface of the CPB circuit, ischemia-reperfusion injury, operative trauma, non-pulsatile blood flow during CPB, and pre-existing left ventricular dysfunction have all been associated with the development SIRS following cardiac surgery [109, 118]. Interleukins (IL-6 and IL-8) and tumor necrosis factor (TNF)-α are thought to be central to the SIRS response [124]. The effect of SIRS upon the kidney is unknown, but in animal models systemic inflammation can result in renal injury [125, 126]. Thus, it is a distinct possibility that CPB- induced SIRS may have a significant deleterious effect upon the kidney.

Microemboli generated by the CPB circuit can also cause directly renal injury. The microemboli are composed of fibrin, platelet aggregates, cellular debris, fat, and air [109]. Although the CPB system can filter larger emboli (greater than 40 μm) smaller emboli are not effectively filtered and can cause direct damage to renal capillaries [118]. Studies using transcranial doppler ultrasound to record emboli counts during CPB found that the number of emboli during CPB was independently associated with CSA-AKI [127].

1.2.6.3 Post-operative events

Postoperative haemodynamic instability, inotrope or pressor use and/or nephrotoxins (such as non-steroidal anti-inflammatory drugs), volume depletion and sepsis/SIRS are all events that may cause or exacerbate AKI [120].

1.2.7 Strategies to Reduce Incidence of CSA-AKI

1.2.7.1 General measures

Pre-operative recognition of CSA-AKI risk is key to improving outcomes. As previously discussed several scoring systems exist to identify patients at risk of CSA-AKI. In at risk patients the pre-operative correction of intravascular volume depletion and optimization of cardiac failure will increase cardiac output and renal perfusion and may reduce the potential for CSA-AKI. Moreover, the discontinuation of nephrotoxins, and potentially delaying surgery for 24 hours after exposure to iodinated contrast media may also help reduce the incidence of CSA-AKI [118].

1.2.7.2 Optimising Cardiopulmonary Bypass

Conceptually, many of the pathophysiologic mechanisms that result in CSA-AKI stem from CPB. The duration of CPB is independently associated with the development of CSA-AKI [128]. Tissue perfusion during CPB is a function of pump flow and arterial resistance. Haemodilution is undertaken during CPB to decrease blood viscosity and improve end organ microcirculatory flow, but haemodilution to a haematocrit of less than 21% is associated with a significant increase in the incidence of CSA-AKI [109]. Blood conservation guidelines published by the Society of Thoracic Surgeons and the Society of Cardiovascular Anaesthesiologists suggest maintaining a hematocrit of at least 21% (haemoglobin concentration, 7 g/dL) during CPB to reduce the incidence of CSA-AKI [129]. Pulsatile flow during CPB may also improve end-organ perfusion and has been shown to preserve renal function better than standard non-pulsatile CPB [118].

The development of 'off-pump' surgical techniques that allow the elimination of CPB and maintenance of pulsatile intra-operative blood flow could theoretically reduce the incidence of CSA-AKI. However, these techniques may be associated with more hemodynamic instability as the left ventricle is compressed when manipulating the heart to access the

coronary arteries [130]. Published studies comparing 'off-pump' and 'on-pump' CABG upon renal outcomes have yielded conflicting results. Most of these studies were single centre, and observational in design, including heterogeneous patient populations with vastly different baseline risk of developing CSA-AKI. Furthermore, they used a variety of definitions of CSA-AKI. A recent meta-analysis of 22 studies (6 RCTs and 16 observational studies) including approximately 28,000 patients, demonstrated a statistically significant reduction in CSA-AKI (OR 0.57; 95% CI 0.43 to 0.76) with 'off-pump' compared to conventional 'on-pump' surgery [131]. Also, Hix and coworkers [132] reported a large cohort of 1365 patients undergoing 'off-pump' CABG matched by propensity score with 1365 patients undergoing conventional 'on-pump' CABG. These investigators found that 'off-pump' surgery was associated with a significant 2-fold reduction in the risk of CSA-AKI and a 2-fold decrease in the need for RRT. Prospective, randomized studies to assess the effect of 'off-pump' surgery upon CSA-AKI are currently underway.

1.2.7.3 Pharmacological interventions to modify the incidence of CSA-AKI

A multitude of clinical studies have investigated the effects of pharmacological renoprotective agents aimed at improving renal perfusion, reducing renal oxygen consumption, or attenuating inflammation to reduce the incidence of CSA-AKI. To date these interventions have been largely unsuccessful. Potential explanations for the failure of these measures to prevent CSA-AKI include:

1. The pathophysiology of CSA-AKI is complex, and most interventions have targeted a single pathophysiological pathway.
2. CSA-AKI is defined by an increase in sCr, or decrease in urine output. These are late events occurring up to 24-48 hours after the renal insult. Therapies to ameliorate CSA-AKI instituted at this late stage are unlikely to be successful.
3. Most clinical trials have enrolled small numbers of low risk patients and are as such underpowered to detect small benefits of trial therapies.

Renal vasodilators: Dopamine, which causes dilatation of both afferent and efferent glomerular arterioles and results in increased renal blood flow, has failed to demonstrate any renoprotective benefit after cardiac surgery [133]. Fenoldopam, a selective dopamine receptor agonist may also be used to increase renal perfusion and has been shown in some studies to reduce CSA-AKI [134-136]. Trials assessing the effect of fenoldapam upon CSA-AKI are limited by small sample size, variation in trial inclusion criteria and fenoldapam

treatment regimes. Furthermore, concerns have been raised that the beneficial renal vasodilation afforded by fenoldopam may be offset by systemic hypotension that results in an overall net reduction in renal blood flow [120]. Currently further evaluation of fenoldopam to prevent CSA-AKI is needed.

Drugs that induce natriuresis: Forced diuresis theoretically may reduce AKI by preventing tubular obstruction and decreasing oxygen consumption through the inhibition of the Na-K-2Cl co-transporter in the loop of Henle. Randomised trials of both frusemide and mannitol have failed to demonstrate any renoprotective effects of forced diuresis [137, 138]. In fact, when combining the results of these trials there is a non-significant trend towards an increase in the need for RRT and incidence of AKI attributable to the use of diuretic agents [133].

Cardiac natriuretic peptides induce natriuresis by increasing glomerular filtration rate and inhibiting sodium reabsorption in the collecting ducts. Following cardiac surgery the administration of recombinant cardiac natriuretic peptides increases urine output and reduces post-operative creatinine levels. Individual RCTs have not detected a consistent reduction in the need for RRT or post-operative mortality with the peri-operative administration of recombinant natriuretic peptides, but as with so many trials in this arena, these studies were small and underpowered [139, 140]. A meta-analysis evaluating the efficacy of natriuretic peptides in patients undergoing cardiac as well as non-cardiac surgery has demonstrated a reduced need for post-operative RRT [133]. An adequately powered study examining natriuretic peptides in cardiac surgery is now needed.

Drugs that block inflammation: Drugs targeting post CPB SIRS to reduce CSA-AKI have been intensively investigated and in majority have yielded disappointing results. For example, statins attenuate inflammation and oxidative stress but do not reduce CSA-AKI [141]. N-acetylcysteine, which has been proven to prevent AKI in animal models, has failed to show the same promise in clinical studies [131].

Other strategies: Calcium channel antagonists***:*** A systematic review of the perioperative administration of calcium channel antagonists (CCAs) in non-cardiac surgery reported that post-operative eGFR was 8.54 mL/min (95% CI 0.82, 16.25, p = 0.03) higher in patients that received CCA compared with patients receiving placebo [142]. Unfortunately, trials in patients undergoing cardiac surgery have not found a significant benefit of CCAs upon post-

operative renal function [133]. The renoprotection of CCAs seen in non-cardiac surgical patients may be offset by CCA induced hypotension in patients with compromised cardiac function undergoing cardiac surgery.

Urinary alkalinization: Hydration plus urinary alkalinization with sodium bicarbonate has been used extensively to prevent contrast-induced nephropathy where it has been associated with inconsistent results. Sodium bicarbonate to prevent CSA-AKI is less well studied. A small randomized trial of 100 patients (receiving normal saline or sodium bicarbonate during cardiac surgery) found that patients receiving sodium bicarbonate peri-operatively were less likely to develop a postoperative increase in sCr >25% from baseline within the first five postoperative days compared with the patients that received normal saline (OR 0.43 [95% CI 0.19-0.98])(p = 0.043) [143]. The role of sodium bicarbonate in the protection of CSA-AKI is currently the focus of a number of larger RCTs.

Remote ischaemic pre-conditioning (RIPC): RIPC is the phenomenon by which the application of brief intervals of non-lethal ischemia to a distant organ provides protection of another organ to a subsequent injurious ischaemic insult. RIPC is reported to reduce renal injury following abdominal aortic aneurysm (AAA) surgery [144]. As the mechanisms of renal ischemia–reperfusion injury following AAA surgery are broadly similar to those proposed for CSA-AKI, and that RIPC may modulate CPB related inflammatory response, RIPC represents an attractive non-invasive therapy to ameliorate CSA-AKI.

In cardiac surgery a retrospective secondary analysis of renal outcomes drawn from 2 randomized trials of RIPC for myocardial protection found that RIPC resulted in a reduction in CSA-AKI [145]. Three small prospective studies in cardiac surgery have prospectively investigated the renoprotective effects of RIPC. Rahman *et al* failed to show a beneficial effect of RIPC upon renal injury following CABG, although renal function was not the primary outcome of this study, and was assessed only upon the fourth post-operative day. Conceivably sCr may have risen and returned to baseline within this time frame [146]. Choi *et al*, also failed to show any renoprotective effect of RIPC within a small heterogeneous population undergoing a variety of complex cardiac surgical procedures [147]. Finally, Zimmerman *et al* found that RIPC significantly reduced the incidence of CSA-AKI in their cohort of 118 patients undergoing cardiac surgery [148]. RIPC needs to undergo further investigation as a renoprotective procedure; larger RCTs are needed, and RIPC must be tested in patients with pre-existing renal disease before it becomes a mainstream therapy.

Prophylactic renal replacement therapy: Prophylactic RRT has been studied in high-risk patients with pre-operative renal dysfunction. In a single study, 44 patients with sCr greater than 221 μmol/L were randomized to either prophylactic RRT or control [149]. In the group receiving RRT, mortality was 4.8% versus 30.4% in the control group, and postoperative AKI requiring RRT was 4.8% versus 34.8% in the control group. Prophylactic RRT may become a viable option in the future, but before this these results will have to be repeated in larger RCTs.

1.2.8 Renal Biomarkers for the early diagnosis of AKI

CSA-AKI is diagnosed by peri-operative changes in sCr level and urine output [111]. Creatinine is the product of creatine breakdown and is filtered by the glomerulus, with blood creatinine levels rising, if glomerular filtration of creatinine is deficient. sCr has several limitations as a biomarker of AKI; firstly creatinine release is affected by age, gender, diet, muscle mass, drugs, and exercise [108]. Secondly, up to 40% of creatinine clearance is due to the renal secretion of creatinine into urine, which could potentially mask important changes in GFR [108]. Finally, sCr levels only become abnormal when greater than 50% of glomerular filtering capacity is lost, and it may require up to 24 hours following AKI for increases in sCr concentration to become detectable [103]. Urine output is also a crude gauge of renal function but may be a more sensitive indicator of changes in renal hemodynamics than a measure of solute clearance. Importantly, many drugs used post-operatively (eg: diuretics, inotropes and vasopressors) will confound the use of urine output as an accurate measure of renal function [103].

Given the limitations of sCr and urine output for the detection of CSA-AKI there is a growing interest in novel biomarkers that allow rapid and reliable diagnosis of kidney injury. Early diagnosis of CSA-AKI may facilitate better peri-operative risk stratification, and allow the institution of renoprotective therapies during a 'time-window' when they will have the best chance of being effective. They may also offer a novel surrogate end-point for trials of renoprotection in cardiac surgery.

The most promising AKI biomarkers to emerge include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-

18) and liver-type fatty acid binding protein (L-FABP). These biomarkers have been evaluated in several AKI populations, but CSA-AKI has attracted most interest as it is the second most common cause of AKI in critically ill patients and patients undergoing cardiac surgery are a relatively homogenous, well-characterized population, and most importantly the timing of renal injury is known.

1.2.9 Summary

AKI is common complication after cardiac surgery and is associated with an increased risk of both morbidity and mortality. Despite recent advances in the understanding of the predisposing risk factors and the pathophysiological processes central to the development of CSA-AKI, neither the incidence nor the mortality associated with the condition have changed. Currently there are no established prophylaxes or therapies for CSA-AKI and novel strategies to prevent and manage this complication are now urgently needed.

1.3 Aims of this thesis

The remainder of this thesis is structured into three parts. The first section describes outcomes of patients undergoing cardiac surgery within Barts Health NHS Trust. I have developed a cardiac surgical dataset that has allowed analysis of our cardiothoracic unit's outcomes. Analysis of this dataset has allowed me to investigate the effect of CKD upon outcome after CABG surgery, and also to study the effect of CSA-AKI following CABG surgery upon patients at our institution.

The second section of this thesis describes a randomized control trial in patients with CKD undergoing CABG surgery. The specific aims of this trial were to assess the potential for remote ischaemic preconditioning to reduce CSA-AKI and myocardial injury in patients with CKD undergoing CABG surgery.

Finally the third section of this thesis describes the development of a panel of renal AKI biomarkers that we hoped would allow the accurate prediction of CSA-AKI in patients with CKD undergoing CABG surgery.

Chapter 2

2. Outcomes of patients with chronic kidney disease undergoing coronary artery bypass graft surgery at Barts Health NHS Trust: 2003 to 2007

Background

Patients with ESRD represent a group at particularly high risk for mortality following CABG. Patients with lesser degrees of CKD undergoing CABG are less well studied. The aim of this study was to evaluate the impact of CKD stage upon mortality in patients undergoing CABG at Barts Health NHS Trust.

Methods

This was an observational cohort study of 4769 patients undergoing CABG at Barts Health NHS Trust between 2003 and 2008. eGFR was calculated from the MDRD equation. The cohort was divided into four groups according to pre-operative eGFR: Reference group (eGFR >90 mL/min), stage 2 CKD (eGFR 89.9-60 mL/min), stage 3 CKD (eGFR 59.9-30 mL/min) and stage 4 CKD (eGFR <30 mL/min). Clinical outcomes were compared between the groups. The primary outcome measure was five-year all cause mortality.

Results

779 deaths occurred during median 5.5 (CI 4.2 to 6.8) years follow-up. Pre-operative eGFR was significantly lower for the patients that died compared with survivors (60.8 vs 72.9 mL/min; $p<0.0001$). Five-year mortality adjusted for potentially confounding factors following CABG was significantly worse for patients with stage 3 CKD (HR 1.29, 95% CI 1.02 to 1.64) and for patients with stage 4 CKD (HR 3.12, 95% CI 2.08 to 4.67) when compared with the reference group.

Conclusions

Patients with stage 3 and 4 CKD represent a group at particularly high risk of adverse outcome following CABG.

2.1 Introduction

In recent years the number of patients with CKD undergoing cardiac surgery has increased dramatically [3, 150, 151]. Current data suggests that CKD may now affect more than one-quarter of all cardiac surgical patients [150, 152]. Although severe CKD and ESRD are understood to be risk factors for adverse outcomes following cardiac surgery, there is limited information upon the risk associated with lesser degrees of CKD. Furthermore, many previous observational studies that have investigated the association between CKD and outcome following cardiac surgery have used arbitrary sCr measurements to define CKD [153-156]. This is inappropriate as sCr measurements can vary by as much as 20% each day due to factors independent of kidney function. In contrast eGFR is a more accurate and robust measure of kidney function that may identify patients with mild CKD despite normal or near normal sCr levels.

This chapter represents an analysis of Barts Health NHS Trust cardiac surgical dataset. The aim of the study was to evaluate the impact of CKD stage defined according to National Kidney Foundation guidelines using eGFR cut-offs upon mortality in patients undergoing CABG at Barts Health NHS Trust.

2.2 Methods

2.2.1 Patients and Setting

Between January 1 2003 and December 31 2007, 4,891 patients underwent isolated first time CABG at Barts Health NHS Trust. The 87 patients who had incomplete surgical datasets and the 35 patients who required renal replacement therapy pre-operatively were excluded, leaving 4,769 patients in the study cohort (**Figure 3**).

2.2.2 Data Collection

Detailed clinical information was recorded prospectively and stored electronically. Baseline clinical data included age, sex, body mass index (BMI), history of MI, PCI, insulin

dependent diabetes mellitus (IDDM), hypertension, peripheral vascular disease (PVD), stroke, and pre-operative left ventricular function, severity of CAD, and the logistic EuroSCORE [157].

Operative data recorded included procedural urgency, use of CPB, internal mammary arterial (IMA) grafts, intra-aortic balloon pump (IABP), cross clamp time, and perfusion time. Procedural urgency was defined as urgent, or elective. Urgent surgery was defined by the requirement for the patient to remain in hospital for CABG following coronary angiography. Elective surgery was defined as CABG in patients who were discharged from the hospital following coronary angiography and readmitted in a planned way for surgery at a later date.

2.2.3 Renal function

sCr concentration was measured pre-operatively in all patients undergoing cardiac surgery. For patients undergoing elective surgery, the sCr concentration was measured in a preadmission clinic approximately 4 weeks before surgery. For patients undergoing urgent surgery, sCr concentration was measured on admission to hospital. eGFR was calculated from the Modification of Diet in Renal Disease equation [158].

sCr was measured daily for the first post-operative week unless patients were discharged within this period. Post-operative AKI was defined as a 50% increase in sCr concentration within seven days of surgery compared with the pre-procedure level.

2.2.4 Outcome Measures

The primary study end point was mid-term all-cause mortality. The secondary end-point was 30-day post-operative mortality. Mortality data were obtained from the United Kingdom Office of National Statistics which periodically links live/death status of treated patients to our unit's surgical database as part of the national Central Cardiac Audit Database. Study patients were followed up until August 2011. The median follow-up period was 5.5 (interquartile range (IQR) 4.2 - 6.9) years.

2.2.5 Ethics

Data were collected routinely as part of a national cardiac surgical audit and patient identifiers were removed prior to analysis. The local ethics committee advised us that formal ethical approval was not required for this study.

2.2.6 Statistical Analysis

Using pre-operative eGFR, and guidelines defined by the National Kidney Foundation [7], the cohort was divided into four groups for analysis; patients with an eGFR > 90 mL/min (Reference group), patients with stage 2 CKD (eGFR 60–89.9 mL/min), patients with stage 3 CKD (eGFR 30-59.9 mL/min) and patients with stage 4 CKD (eGFR < 30 mL/min).

Baseline clinical characteristics and procedural data were compared between the groups. Categorical data are summarised using absolute values (percentage). Continuous data with a normal distribution are presented as mean ± standard deviation or, where skewed, as median (IQR). Normally distributed data were compared with a one-way analysis of variance (ANOVA) and non-normally distributed variables were compared with the Kruskal-Wallis test. Categorical data were compared using the Pearson chi square test. Procedural outcomes, 30-day mortality, and mid-term mortality were compared between the four groups. Potential independent associations with 30-day mortality were assessed using multivariate logistic regression. Mid-term survival was described using the Kaplan-Meier method, and comparisons between groups were made using the log-rank statistic. Variables associated with mid-term mortality were analysed using multivariable Cox proportional regression in order to account for confounding factors. Statistical analyses were performed using SPSS (Version 18.0, IBM SPSS statistics UK).

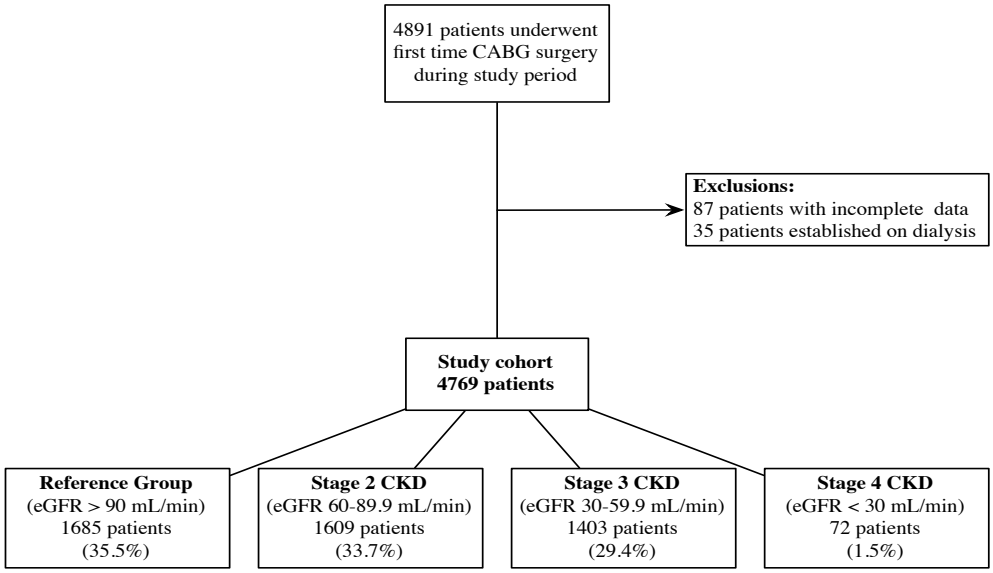


Figure 3 Flow diagram of patients treated by CABG between January 2003 and December 2007 describing patient exclusions and pre-operative stage of chronic kidney disease.

2.3 Results

2.3.1 Patient clinical and procedural characteristics

The baseline clinical characteristics of the study groups are shown in **Table 6**. Among 4769 patients who underwent CABG, 1609 (33.7%) patients had CKD stage 2, 1403 (29.4%) patients had CKD stage 3 and 72 (1.5%) patients had CKD stage 4. Patients with more advanced CKD were older, more likely to be female and had a lower BMI. When compared with patients with an eGFR > 60 mL/min (reference group and stage 2 CKD group combined), patients with stage 3 or 4 CKD were significantly more likely to have had a prior MI, PCI, stroke, PVD, left ventricular impairment, three vessel or left main stem CAD and had higher median logistic EuroSCORE. Also these patients were more likely to undergo urgent surgery and less likely to receive an IMA graft. Patients with CKD stage 4 were most likely to have IDDM.

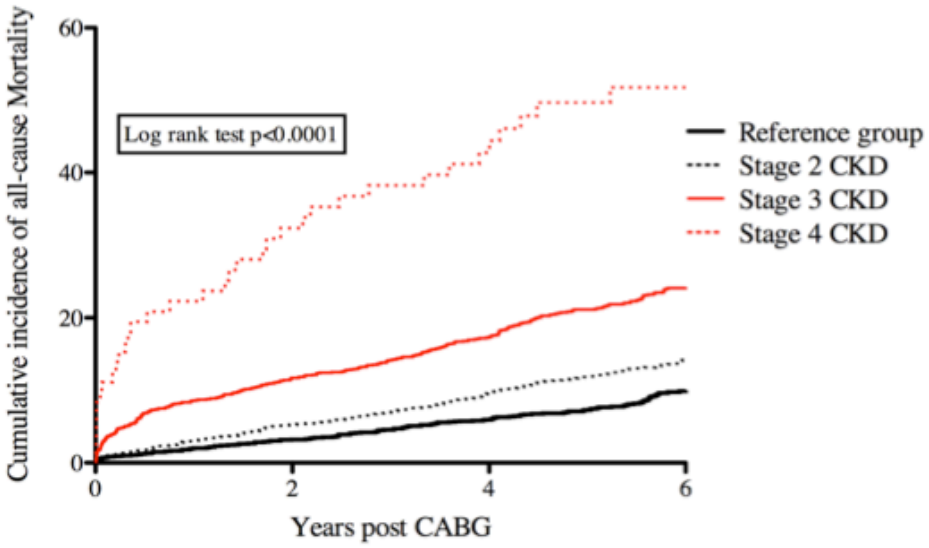
2.3.2 Post-operative complications and 30-day mortality

The in-hospital complications and 30-day mortality data are also shown in **Table 6**. AKI developed in 613 (12.9%) patients. The rate of post-operative AKI and dialysis were highest in patients with stage 4 CKD. Length of hospital stay was also significantly longer in this group. The 30-day mortality rates were 0.8% (reference group), 0.9% (stage 2 CKD), 3.1% (stage 3 CKD), and 11.1% (stage 4 CKD) ($p<0.0001$). Logistic regression confirmed both stage 3 and stage 4, but not stage 2 CKD as univariate predictors of 30-day mortality post CABG, when compared to reference group (**Table 7**). After multivariable adjustment for all factors with a significant ($p<0.15$) univariate relationship with 30-day mortality after CABG (see **Table 7**) only stage 4 CKD independently predicted 30-day mortality (when compared to reference group). In a multivariate model that included just CKD stage and logistic EuroScore both stage 3 and stage 4 CKD were significant predictors of 30-day mortality. Other independent predictors of 30-day mortality included increasing age, hypertension and need for pre-operative IABP.

2.3.3 Mid-term all-cause mortality

Kaplan-Meier analysis (**Figure 4**) showed significant differences between the study groups in mortality rates during follow-up after CABG. The one-year mortality rates in the reference group, stage 2 CKD group, stage 3 CKD group and stage 4 CKD group were 2.0% (95% CI 0.4 – 6.2), 3.1% (95% CI 1.0 – 7.2), 8.6% (95% CI 5.3 – 12.8) and 22.3% (95% CI 8.6 – 39.9), respectively. The five-year mortality rates were 7.3% (95% CI 4.3 – 11.3), 11.9% (95% CI 8.5 – 15.9), 21.1% (95% CI 17.4 – 25.1) and 49.7% (95% CI 37.1 – 61.1)

respectively. Cox-regression analysis demonstrated a significant univariate relationship between CKD stages 2, 3 and 4 and mid-term mortality when compared to the reference group. The relationship between stage 3 and stage 4 CKD, but not stage 2 CKD, and mid-term mortality following CABG persisted after multivariate analysis (**Table 7**). Other independent predictors of mid-term mortality included increasing age, presence of IDDM, PVD, left ventricular ejection fraction less than 50%, and the need for urgent CABG surgery.



Numbers at risk	Years post CABG						
	0	1	2	3	4	5	6
Reference group	1685	1645	1609	1585	1383	1010	691
Stage 2 CKD	1609	1551	1499	1466	1307	1034	689
Stage 3 CKD	1403	1276	1212	1176	1049	792	533
Stage 4 CKD	72	55	47	43	35	25	16

Figure 4 Kaplan-Meier curve describing mid-term mortality after CABG surgery in relation to CKD stage

Table 6 Clinical and procedural characteristics of study cohort according to CKD stage

	eGFR, mL/min				
	Reference > 90 (n=1685)	**CKD stage2 60 – 89.9 (n=1609)**	**CKD stage 3 30 – 59.9 (n=1403)**	**CKD stage 4 < 30 (n=72)**	**p value**
Patient Characteristics					
Age	59.7 ± 8.7	68.0 ± 7.4	73.7±6.6	73.8±9.3	<0.0001
Gender, female	193 (11.5)	300 (18.6)	456 (32.5)	27 (37.5)	<0.0001
BMI	30.0 ± 5.7	27.4 ± 4.9	25.8±4.5	24.4±3.6	<0.0001
Hypertension	1291 (76.6)	1277 (79.4)	1110 (79.1)	56 (77.8)	0.217
IDDM	164 (9.7)	148 (9.2)	164 (11.7)	14 (19.4)	0.007
Previous MI	749 (44.5)	707 (43.9)	687 (49.0)	36 (50.0)	0.022
Previous PCI	217 (12.9)	165 (10.3)	82 (5.8)	6 (8.3)	<0.0001
Previous stroke	88 (5.2)	109 (6.8)	125 (8.9)	7 (9.7)	0.001
PVD	160 (9.5)	193 (12.0)	242 (17.2)	21 (29.2)	<0.0001
LVEF < 50%	584 (34.7)	600 (37.3)	587 (41.7)	40 (55.6)	<0.0001
3 vessel coronary disease	1136 (67.4)	1067 (66.3)	997 (70.9)	52 (72.2)	0.030
Left main stem disease	428 (25.4)	376 (23.4)	400 (28.5)	21 (29.2)	0.012
Procedural Characteristics					
Procedural urgency	523 (31.0)	481 (29.9)	528 (37.6)	29 (40.3)	<0.0001
CPB	1560 (92.6)	1492 (92.7)	1291 (92.0)	67 (93.1)	0.889
IMA usage	1579 (93.7)	1490 (92.6)	1232 (87.8)	62 (86.1)	<0.0001
Preoperative IABP	19 (1.1)	21 (1.3)	20 (1.4)	3 (4.2)	0.166
Cross-clamp time	57.3±22.3	55.4±20.1	54.6±24.7	55.8±18.6	0.690
Perfusion time	83.4±40.8	82.1±40.6	80.7±36.8	87.3±37.1	0.216
EuroSCORE	1.6 (1.1 to 2.7)	2.7 (1.7 to 4.7)	4.3 (2.9 to 7.9)	8.6 (4.3 to 9.1)	<0.0001
Post operative outcomes					
AKI	157 (9.3)	176 (10.9)	254 (18.1)	26 (36.1)	<0.0001
Post-operative dialysis	7 (0.4)	9 (0.6)	18 (1.3)	6 (8.3)	<0.0001
Post-operative stroke	4 (0.2)	11 (0.7)	12 (0.9)	0 (0)	0.106
Length of hospital stay	7 (6 to 11)	8 (6 to 13)	9 (6 to 16)	12 (7 to 26)	<0.0001
30-day mortality	13 (0.8)	15 (0.9)	43 (3.1)	8 (11.1)	<0.0001

Table 7 Relation of 30-day and mid-term mortality to CKD stage

Of	eGFR, mL/min			
	Reference group > 90 (n=1685)	**CKD stage 2 60 – 89.9 (n=1609)**	**CKD stage 3 30 – 59.9 (n=1403)**	**CKD stage 4 < 30 (n=72)**
30-day mortality	13 (0.8)	15 (0.9)	43 (3.1)	8 (11.1)
Unadjusted OR (95% CI)	1	1.21 (0.57–2.55)	4.07 (2.18 – 7.59)	16.08 (6.44 – 40.16)
OR adjusted for EuroScore (95% CI)	1	1.08 (0.51–2.29)	3.07 (1.62 – 5.82)	8.21 (3.03 – 22.24)
Multivariate adjustment* (95% CI)	1	0.82 (0.37–1.79)	1.93 (0.92 – 4.05)	6.67 (2.36 – 18.86)
Mid-term mortality	159 (9.4)	240 (14.9)	343 (24.4)	37 (51.4)
Unadjusted HR (95% CI)	1	1.57 (1.28–1.92)	2.81 (2.33 – 3.39)	8.15 (5.69 – 11.65)
Cox multivariate adjustment §(95% CI)	1	1.01 (0.81–1.26)	1.29 (1.02 – 1.64)	3.12 (2.08 – 4.67)

eGFR indicates estimated glomerular filtration rate; OR odds ratio; HR hazard ratio.

* Multivariate logistic regression analysis included the following variables: age, previous MI, hypertension, previous stroke, PVD, presence of three vessel CAD, presence of left main stem CAD, pre-operative left ventricular function, procedural urgency, use of IMA, use of peri-operative IABP, and CKD stage.

§ Multivariate logistic regression analysis included the following variables: age, BMI, previous MI, hypertension, previous stroke, PVD, presence of three vessel CAD, presence of left main stem CAD, pre-operative left ventricular function, procedural urgency, use of CPB, use of IMA, use of peri-operative IABP, and CKD stage.

2.4 Discussion

These data demonstrate the relationship between CKD stage and outcome after CABG. Consistent with previous research we have found that CKD is a harbinger of poor outcome following CABG. In this surgical cohort stage 3 CKD or worse (defined as an eGFR < 60 mL/min) was common, affecting approximately one third of patients, with these patients accounting for a disproportionate two thirds of all deaths within the first 30-days of surgery. Within 5-years of surgery 22% of patients with CKD stage 3 or stage 4 were dead, in comparison with a 5-year mortality rate of only 12% of patients with a pre-operative eGFR > 60mL/min.

The association between renal dysfunction and adverse prognosis after cardiac surgery is well described. However, most previous observational studies have defined kidney disease on the basis of arbitrary measurements of sCr, which may underestimate the prevalence of significant kidney disease [153-156]. For example, one commonly used definition of kidney disease used was sCr greater than 1.5 mg/dL (133 μmol/L). If this definition was applied to our cohort only 7.4% of patients would be considered to have kidney disease. eGFR is a far more discriminatory measure of renal function, that allows the detection of patients with less severe kidney disease. GFR has now been confirmed as powerful independent predictor of cardiovascular events and mortality in a number of settings, including after coronary surgery, PCI and MI [150, 152, 159, 160]. This data adds to this rapidly growing evidence base, but most importantly it highlights the risk faced by patients with stage 3 and stage 4 CKD undergoing CABG. Novel strategies to reduce risk in this group of patients are now urgently needed.

In keeping with previous research we found that the relationship between mortality risk after coronary surgery and kidney dysfunction is non-linear [150]. The risk of mortality for patients with mild kidney disease (stage 2 CKD) appears broadly similar to patients in our reference group. Once patients develop stage 3 CKD mortality risk following surgery seems to increase steeply.

Although we have no data upon cause of death in our cohort, similar studies have reported that the majority of deaths following coronary surgery in patients with CKD are due to cardiovascular disease [152]. There are a number of potential explanations for the increase in cardiovascular mortality observed in patients with CKD. Firstly, as previously described,

traditional cardiovascular risk factors such as diabetes mellitus and hypertension, themselves independently associated with an increased mortality risk, are more prevalent in patients with CKD [161]. Secondly, the uraemic milieu that characterizes CKD is associated with novel cardiovascular risk factors such as inflammation, endothelial dysfunction and oxidative stress, hyperhomocysteinaemia and vascular calcification [161]. These novel risk factors may interact with traditional cardiovascular risk factors to accelerate arterial disease and result in the development of *de novo* cardiovascular events. An excess of MI and stroke has been observed in patients with CKD following cardiac surgery [151]. Thirdly, left ventricular hypertrophy, dilatation and dysfunction are more common in patients with significant CKD and are associated with increased mortality in both population studies and following coronary surgery [96]. Fourthly, patients with significant CKD are significantly more likely to sustain peri-operative AKI, which is also an independent predictor of cardiovascular risk and mortality after cardiac surgery [6]. Finally, patients with CKD are less likely to receive prognostically beneficial cardiovascular risk modification therapies such as ACE inhibitors, angiotensin II receptor blockers or aldosterone antagonists[162]. Although these therapies have never been proven within a specific cohort of patients with CKD, there is no reason to expect these therapies to be less efficacious in these patients.

Renal function is often considered a 'barometer' of vascular health [163], and thus CKD is commonly associated with the development of premature arterial disease. In this cohort patients with more advanced CKD were older, had a higher burden of pre-operative comorbidity and thus had an elevated procedural risk, and less commonly received an IMA graft. These differences potentially confound the association between mortality and CKD stage that was identified in this study. After accounting for the differences between patient groups, the presence of CKD stage 3 was associated with an approximately 29% increase in the risk of five-year mortality, while the CKD stage 4 more than tripled the risk compared to patients in the reference group.

In this surgical cohort the overall prevalence of diabetes mellitus (DM) was 31.1%. In patients with CKD the prevalence of DM was 31.7%, although there was a higher prevalence of insulin dependent DM within these patients when compared with patients without CKD. DM is associated with the development of accelerated diffuse CAD. The combination of CKD and DM serves to amplify vascular risk resulting in premature accelerated CAD [164]. Much of the increased morbidity and mortality associated with both DM and CKD is driven by complications of coronary artery disease (CAD) [165, 166].

2.4.1 Comparisons with previous studies

An analysis of the Society of Thoracic Surgeons National Adult Cardiac Database that included approximately 500,000 patients undergoing isolated CABG stratified by eGFR reported 30-day mortality was 1.3% for patients with an eGFR > 90mL/min compared with 1.8%, 4.3% and 9.3% for patients with CKD stage 2, stage 3 and stage 4 respectively. This is broadly similar to the early mortality observed in our CABG cohort.

There is limited data upon long-term outcomes of patients with CKD (defined by eGFR) after CABG. A Swedish study of more than 6,000 patients [151] reported five-year mortality rates of 5.7% for patients with an eGFR > 90mL/min compared with 8.9%, 14.9% and 45.6% for patients with CKD stage 2, stage 3 and stage 4 respectively.

Early outcomes of patients with CKD established upon dialysis have been extensively studied [3, 167]. These patients have a particularly poor prognosis with an operative mortality as high as 9.0% [3]. These patients were excluded form this study in order to maintain the consistency of this thesis which investigates outcomes after cardiac surgery in patients with non-dialysis dependent CKD. Furthermore only 35 patients with dialysis dependent CKD underwent CABG during the study period. Analysis of such a small patient group may yield inconsistent results.

2.5 Strengths and Limitations

This study assessed short- and mid-term outcomes in a large contemporary cohort of consecutive patients who underwent CABG. The only exclusion was pre-operative dialysis requirement and the primary study end-point was all-cause mortality tracked by the Office for National Statistics, a robust clinical outcome. The results are therefore likely to offer genuine insights into the relation between CKD stage and mortality after CABG.

This study suffers from the same limitations as all observational studies. Also, it is an analysis of a single cardiac centers' data, and so the results may not be generalizable to all. Furthermore the primary outcome is all-cause mortality rather than cardiac death, and I acknowledge that the development of new co-morbidities during follow-up might affect long-term prognosis and that an analysis of these might yield interesting results. The hub and spoke model of care employed by cardiothoracic centres in the United Kingdom (and most other

countries) means that patients are typically discharged to their local hospitals and general practitioner after surgery which makes the comprehensive collection of new data during long-term follow-up impossible. For these reasons, rarely are data upon the development of new comorbidities or cause of death available for presentation in cohort studies of this nature.

I have already acknowledged that there were significant differences in baseline and operative characteristics between patient groups. Specifically, patients with more advanced CKD, were older and had a higher burden of comorbidities than other patients. This is the reality of these patients. However, it should be noted that the GFR was estimated with the MDRD equation. The equation uses sCr age and gender to calculate eGFR and so in elderly women eGFR may seem inappropriately low. In this study the elderly and women become more frequently as GFR decreases. Prospective data collection together with multivariate analyses allowed the mitigation of the effect of confounding on the relationship between CKD stage and mortality. However, there remains the possibility of residual confounding from unmeasured variables. For example, the type of medical therapy received by patients following hospital discharge. Patients with CKD are less likely to receive prognostically beneficial medical therapies which may in turn affect long-term outcomes [162].

In this analysis, CKD was defined by a single measurement of eGFR. We did not record whether or not proteinuria was present. Proteinuria is a well-established independent predictor of cardiovascular mortality [21] and has been shown to be an independent predictor of adverse prognosis in diabetic patients undergoing CABG [168]. Data upon proteinuria may have led to the reclassification of a number of patients in the reference group as having CKD. Furthermore, the definition of CKD in this way did not allow us to identify patients who might have had transient AKI following acute myocardial infarction complicated by left ventricular dysfunction or following recent coronary angiography, a clinical variable which is known to affect prognosis adversely following cardiac surgery. In our cohort, most of the patients were elective cases without any reason for AKI.

Despite these limitations the current study highlights the important association between CKD and post-operative outcomes after cardiac surgery. Patients with significant CKD appear to be at particularly high-risk of premature mortality. Despite the high prevalence of CKD in patients undergoing cardiac surgery, and their consistently poor outcomes, these patients are rarely the included in studies of new cardiovascular therapies. As such many treatments routinely used in practice are untested in CKD. Novel therapies to improve outcomes for this

high-risk patient group are urgently needed. This thesis describes my investigations of novel strategies to improve outcomes for patients with CKD undergoing cardiac surgery.

2.6 Conclusions

These data highlights the important relationship between CKD stage, measured by eGFR, and outcome after coronary surgery. Patients with stage 3 and stage 4 CKD represent a group at particularly high risk of adverse outcome following CABG, and targeted research in this group is urgently needed.

Chapter 3

3. Acute Kidney Injury and Mid-Term Outcomes Following CABG: A Matched Propensity Score Analysis

Background

The development of AKI following CABG is associated with increased short- and long-term mortality. Whether AKI has a causal relationship with subsequent mortality or whether the development of AKI simply occurs in patients with more comorbidity, undergoing more complex procedures remains unresolved.

Methods

This was an observational cohort study of 4694 patients who were discharged from hospital after first time CABG surgery at Barts Health NHS Trust between 2003 and 2008. The cohort was dived in those patients that sustained AKI and those that did not. AKI was defined using the RIFLE criteria, which requires at least a 50% increase in serum creatinine. The primary outcome measure was five-year all-cause mortality.

Results

562 (12.0%) patients developed AKI following CABG surgery. Patients that developed AKI were older, more likely to be female and had more associated comorbidity than patients that did not develop AKI. After Cox multivariable analysis the development of AKI was an independent predictor of long-term mortality HR 1.72, 95% CI 1.43-2.07). Subsequently a comparison of 562 patients that sustained AKI with 562 propensity-score matched patients that did not sustain AKI was undertaken. After propensity matching, baseline clinical and operative characteristics were similar between both groups. After Cox multivariable analysis of the propensity-matched cohort AKI remained an independent predictor of long-term mortality (HR 1.52, 95% CI 1.20–1.94).

Conclusions

The development of AKI following CABG is a serious event associated with worse long-term survival. This excess mortality cannot be explained simply by coexisting comorbidity and surgical complexity.

3.1 Introduction

AKI is a common problem following cardiac surgery. Depending upon definition, AKI may complicate more than 30% of cardiac operations [99], with 1-6 % of patients subsequently requiring post-operative dialysis [4, 100]. It is likely that the aetiology of AKI after cardiac surgery is multifactorial with advanced age, pre-existing kidney disease, left ventricular impairment, and procedural complexity with prolonged cardiopulmonary bypass (CPB) and aortic cross times being important predictors of its subsequent development [4, 100, 106, 155].

The need for dialysis after cardiac surgery is a serious event associated with a marked increase in early morbidity and mortality [4, 100]. Recently evidence has emerged to suggest that AKI defined by smaller post-operative increases in sCr is also associated with a poor prognosis following cardiac surgery [113, 169]. This evidence is primarily derived form retrospective analyses of large cardiac surgical datasets or observational studies and may be confounded by the fact that patients with the highest burden of comorbidity before surgery or those undergoing the most complex operations are most likely to develop post-operative AKI. By virtue of comorbid disease or procedural complexity these patients are likely to have a worse post-surgical prognosis independent of the development of AKI. Whether AKI has a causal relationship with subsequent mortality or whether the development of AKI simply reflects comorbidity and/or procedural complexity within a high-risk patient population remains unresolved.

This chapter represents an analysis of Barts Health NHS Trust cardiac surgical dataset with the aim of evaluating the impact of AKI upon survival of patients following CABG. Using propensity score matching I have minimized the impact of patient comorbidity and surgical complexity upon long-term mortality after cardiac surgery.

3.2 Methods

3.2.1 Patients and Setting

This study analyses consecutive patients undergoing isolated first time CABG surgery at Barts Health NHS Trust who survived to hospital discharge. During the study period of January 1 2003 to December 31 2007 4891 patients underwent first time CABG surgery, of whom we excluded 87 patients with incomplete surgical database records, 35 patients established upon pre-operative renal replacement therapy and 75 patients that died in hospital post-operatively. The final study cohort comprised 4694 patients who for subsequent analysis were divided into two groups; those that developed AKI (AKI group; n=562) and those that did not develop AKI (no AKI group; n=4132) (**Figure 5**).

3.2.2 Data Collection

Detailed clinical information was recorded prospectively and stored electronically. Baseline clinical data included age, sex, BMI, symptom status (NYHA class and CCS angina status), history of MI, PCI, pre-operative atrial fibrillation, hypertension, diabetes mellitus, PVD, stroke, chronic obstructive pulmonary disease (COPD), baseline renal function (sCr and eGFR) and pre-operative left ventricular function, and severity of CAD.

Procedural information recorded included procedural urgency, use of CPB, internal IMA grafts, use of IABP, cross clamp time, and perfusion time. Procedural urgency was defined as urgent, or elective. Urgent surgery was defined by the requirement for the patient to remain in hospital for CABG following coronary angiography. Elective surgery was defined as CABG in patients who were discharged from the hospital following coronary angiography and readmitted in a planned way for surgery at a later date.

3.2.3 Definition of AKI

sCr concentration was measured pre-operatively in all patients undergoing cardiac surgery. For patients undergoing elective surgery, the sCr concentration was measured in a preadmission clinic approximately 4 weeks before surgery. For patients undergoing urgent surgery, sCr concentration was measured on admission to hospital. eGFR was calculated from the Modification of Diet in Renal Disease equation [158].

sCr was measured daily for the first post-operative week unless patients were discharged within this period. AKI was defined according to the RIFLE classification by the change in

sCr post-operatively compared with baseline sCr. The RIFLE classification system requires sCr levels to increase by at least 50% from baseline to be classified as AKI [110]. Patients sustaining AKI were further stratified according to RIFLE class: RIFLE-R corresponds to a 50% increase in sCr, RIFLE-I to a 100% increase in sCr, and RIFLE-F to a 200% increase in sCr.

3.2.4 Outcome Measures

The primary study end point was mid-term all-cause mortality. Mortality data were obtained from the United Kingdom Office of National Statistics which periodically links live/death status of treated patients to our unit's surgical database as part of the national Central Cardiac Audit Database. Study patients were followed up until August 2011. The median follow-up period 5.6 years (IQR 4.2 to 6.9). In addition, early in-hospital outcomes including peri-operative stroke, need for resternotomy, 30-day mortality and length of hospital stay were also recorded.

3.2.5 Ethics

Data were collected routinely as part of a national cardiac surgical audit and patient identifiers were removed prior to analysis. The local ethics committee advised us that formal ethical approval was not required for this study.

3.2.6 Statistical Analysis and Propensity Score Matching

All Statistical analyses were performed using SPSS (Version 18.0, IBM SPSS statistics UK). Baseline clinical characteristics and procedural data were compared between the groups. Categorical data are summarised using absolute values (percentage). Continuous data with a normal distribution are presented as mean ± standard deviation or, where skewed, as median (IQR). Normally distributed continuous variables are compared using Student's t-tests and the Mann-Whitney U test was used to compare non-normally distributed continuous variables. Categorical data were compared using the Pearson chi-square test.

Baseline comorbidity was unbalanced between the AKI and no AKI groups. A non-parsimonious logistic regression model with AKI as the dependent variable (c-statistic 0.775) was constructed to adjust for the confounding of baseline comorbidity. Covariates in the model included (age, gender, body mass index, previous MI, hypertension, previous stroke, peripheral vascular disease, 3-vessel coronary disease, left main stem disease, LV

ejection fraction <35%, diabetes mellitus, chronic obstructive airways disease, IABP use, IMA use, new stroke, baseline SCr, pre-operative atrial fibrillation, and procedural urgency). To balance comorbidity between the study groups, a greedy matching SPSS macro was used to match the 562 patients that sustained AKI with 562 patients from the 'no AKI' group with similar comorbidity. This created a 'propensity matched no AKI' population.

Mid-term survival was described using the Kaplan-Meier method, and comparisons made using the log-rank statistic. Estimations of risk were calculated using Cox regression analysis. Potential independent predictors of outcome were identified by univariate Cox regression analyses, and all significant univariate predictors ($p \leq 0.15$) were then entered into multivariate Cox regression model.

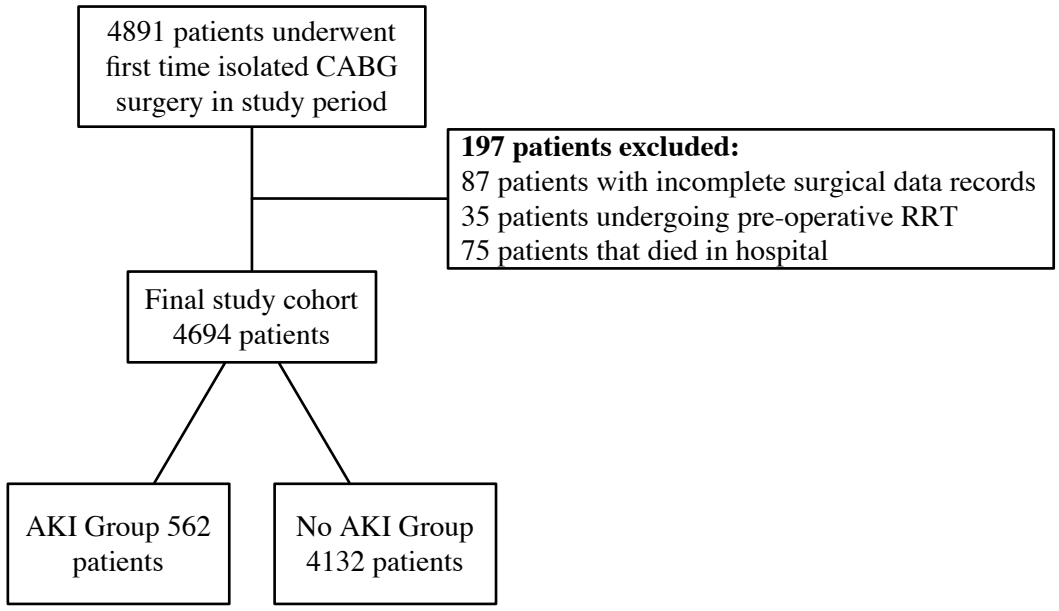


Figure 5 Flow diagram of patients treated by CABG between January 2003 and December 2007 describing patient exclusions and incidence of AKI. RRT indicates renal replacement therapy

3.3 Results

A total of 4694 patients underwent first time CABG surgery and survived to hospital discharge during the study period. A total of 562 patients (12.0%) developed an episode of

post-operative AKI: of these 342 (7.3%) had RIFLE-R, 145 (3.1%) had RIFLE-I, and 75 (1.6%) had RIFLE-F.

3.3.1 Patient and operative characteristics (Table 8)

a) Full unmatched study population

Patients that developed AKI were older (69.4 vs 66.3 years, p<0.0001), more likely to be female (23.7% vs 20.0%, p=0.041) and more likely to have associated comorbidity than patients that did not develop AKI. Notably, patients that developed AKI had more severe pre-operative angina symptoms (CCS class 3-4) (41.5% vs 34.9%, p=0.002), and pre-operative heart failure symptoms (NYHA class 3-4) (30.1% vs 18.8%, p<0.0001). Moreover, DM (46.8% vs 28.8%, p<0.0001), hypertension (86.8% vs 77.0%, p<0.0001), PVD (20.1% vs 11.8%, p<0.0001), previous MI (53.9% vs 44.3%, p<0.0001), LV dysfunction (LVEF<50%) (48.9% vs 36.0%, p<0.0001), pre-operative atrial fibrillation (4.6% vs 2.4%, p=0.003), COPD (11.9% vs 7.9%, p=0.001) and previous stroke (9.9% vs 6.5%, p=0.006) were significantly more common in the AKI group than the no AKI group.

Patients in the AKI group were also more likely to have undergone urgent CABG (43.8% vs 31.0%, p<0.0001), have had a pre-operative IABP (2.8% vs 1.0%, p<0.0001) and were less likely to have received an IMA graft at time of surgery (89.1% vs 91.9%, p=0.026) than patients in the no AKI group.

b) Propensity matched population

After propensity matching, all baseline patients characteristics were balanced between the two groups, except the presence of LMS disease, which was more common in the propensity matched no AKI group (30.6% vs 24.7%, p=0.028). Procedural characteristics were also balanced between the two groups after matching.

3.3.2 Renal characteristics (Table 8)

a) Full unmatched study population

The mean baseline sCr and eGFR for all patients were 99.0 ± 30.1 mmol/L and 75.0 ± 26.4 ml/min respectively. Patients that developed AKI had significantly higher baseline sCr (109.6 vs 97.2 mmol/L, p<0.0001) and lower baseline eGFR (68.9 vs 75.9 mL/min, p<0.0001) than patients that did not develop AKI. Peak post-operative sCr was higher in the

AKI group (228.1 vs 107.8 mmol/L, p<0.0001). Only 21 (3.7%) of the patients with AKI required post-operative dialysis.

b) Propensity matched population

Baseline SCr and eGFR were not different between AKI and no AKI groups after propensity matching. Obviously, peak post-operative SCr remained different between the groups.

3.3.3 Early Postoperative Outcomes in full unmatched population (Table 8)

a) Full unmatched study population

Post-operative stroke (1.4% vs 0.3%, p<0.0001), need for re-sternotomy (5.5% vs 1.8%, p<0.0001) and 30-day mortality (1.1% vs 0.1%, p<0.0001) were significantly higher in the patients that developed AKI. Furthermore, length of hospital stay was longer for patients that developed AKI compared to patients that did not (14 vs 7 days, p<0.0001).

b) Propensity matched population

After propensity matching there was no difference in post-operative stroke, need for resternotomy or 30-day mortality. Length of hospital stay remained longer for patients that developed AKI compared with patients that did not (14 vs 8 days, p<0.0001).

3.3.4 Long Term All Cause Mortality following CABG

Patients that sustained AKI had higher mortality rates during follow-up [1 year 8.6% (95% CI 3.8 – 15.7) and 5 year 24.2% (95% CI 18.4 - 30.4)] than patients that did not sustain AKI [1 year 2.4% (95% CI 1.2 – 4.7) and 5 year 10.6% (95% CI 8.5 – 13.1))(Log rank p<0.0001] (**Figure 6**). After propensity matching long-term mortality remained higher in the AKI group than the propensity matched no AKI group [1 year mortality 5.0% (95% CI 1.3 - 12.6) and 5 year mortality 17.3% (95% CI 11.6 - 24.0))(Log rank p=0.0008] (**Figure 6**).

3.3.5 Predictors of mid-term all-cause mortality following CABG

The development of AKI (HR 1.72, 95% CI 1.43–2.07), age (HR 1.06, 95% CI 1.05–1.07), urgent surgery (HR 1.37, 95% CI 1.16–1.62), baseline sCr (HR 1.54, 95% CI 1.34-1.79) and pre-operative co-morbidities (DM (HR 1.33, 95% CI (1.13–1.56)), COPD (HR 1.61, 95% CI 1.30–1.99), LV ejection fraction < 35% (HR 2.04, 95% CI 1.63–2.54), 3 vessel coronary artery disease (HR 1.20, 95% CI 1.00-1.44) and pre-procedural atrial fibrillation (HR 1.47,

95% CI 1.06–2.06)) were all associated with a significantly increased risk of mid- term post-operative all-cause mortality in the unmatched analysis (**Table 9**). After propensity score matching, development of AKI remained an independent predictor associated with increased mid-term mortality risk (HR 1.52, 95% CI 1.20–1.94) (**Table 9**).

Table 8 Clinical and procedural characteristics of both the unmatched and propensity matched cohorts according to the development of AKI

	No AKI n=4132	AKI n=562	P value	Propensity matched no AKI n=562	P value†
Patient Characteristics					
Age	66.34±9.59	69.36±9.17	<0.0001	69.32±8.92	0.937
Gender, female	825 (20.0)	133 (23.7)	0.041	149 (26.5)	0.271
BMI	27.72±5.31	28.50±5.85	0.001	28.62±6.20	0.740
CCS 3-4	1142 (34.9)	233 (41.5)	0.002	250 (44.5)	0.306
NYHA 3-4	778 (18.8)	169 (30.1))	<0.0001	150 (26.7)	0.209
Hypertension	3182 (77.0)	48 (86.8)	<0.0001	492 (87.5)	0.721
Diabetes Mellitus	1188 (28.8)	263 (46.8)	<0.0001	281 (50.0)	0.281
Previous MI	1830 (44.3)	303 (53.9)	<0.0001	293 (52.1)	0.550
MI within 30 days	467 (11.3)	115 (20.5)	<0.0001	117 (20.8)	0.677
Previous PCI	419 (10.1)	45 (8.0)	0.102	61 (10.9)	0.112
COPD	326 (7.9)	67 (11.9)	0.001	67 (11.9)	1.00
Pre-operative atrial fibrillation	101 (2.4)	26 (4.6)	0.003	28 (5.0)	0.780
Previous stroke	267 (6.5)	54 (9.6)	0.006	58 (10.3)	0.690
Peripheral vascular disease	489 (11.8)	113 (20.1)	<0.0001	111 (19.8)	0.881
LV ejection fraction			<0.0001		0.411
35-50%	1225 (29.6)	213 (37.9)		198 (35.2)	
<35%	264 (6.4)	62 (11.0)		55 (9.8)	
3 vessel coronary disease	2781 (67.3)	415 (73.8)	0.002	417 (74.2)	0.892
Left main stem disease	1058 (25.6)	139 (24.7)	0.656	172 (30.6)	0.028
Procedural Characteristics					
Procedural urgency	1281 (31.0)	246 (43.8)	<0.0001	251 (44.7)	0.764
CPB	3817 (92.4)	526 (93.6)	0.303	528 (93.6)	0.805
Perfusion time	81.07±40.29	84.02±39.21	0.246	80.32±37.10	0.233
Cross clamp time	55.83±22.54	55.77±22.15	0.963	56.55±20.60	0.602
IMA usage	3798 (91.9)	501 (89.1)	0.026	489 (87.0)	0.269
Preoperative IABP	40 (1.0)	16 (2.8)	<0.0001	15 (2.7)	0.855
Renal Characteristics					
Baseline sCr	98.1±27.4	109.6±51.3	<0.0001	107.0±51.3	0.388
Baseline eGFR	75.90±26.00	68.60±28.52	<0.0001	68.46±24.69	0.931
Peak sCr	107.8±36.2	228.1±109.6	<0.0001	122.0±67.2	<0.0001
Post-op dialysis	0 (0)	21 (3.7)	<0.0001	0 (0)	<0.0001
Post operative outcomes					
New stroke	12 (0.3)	8 (1.4)	<0.0001	4 (0.7)	0.246
Return to theatre	75 (1.8)	31 (5.5)	<0.0001	29 (5.2)	0.791
30-day mortality	6 (0.1)	6 (1.1)	<0.0001	1 (0.2)	0.058
Length of stay	7(6 to 11)	14 (8 to 27)	<0.0001	8 (6 to 14)	<0.0001

BMI indicates body mass index; CCS Canadian Cardiovascular Society; NYHA New York Heart Association; MI myocardial infarction; PCI percutaneous coronary intervention; COPD chronic obstructive pulmonary disease; CPB cardiopulmonary bypass; IMA internal mammary artery; IABP intra-aortic balloon pump; sCr serum creatinine; eGFR estimated glomerular filtration rate.

Table 9 Independent predictors of long-term mortality in the unmatched and the matched study cohorts

A) Unmatched population: Multivariate analysis

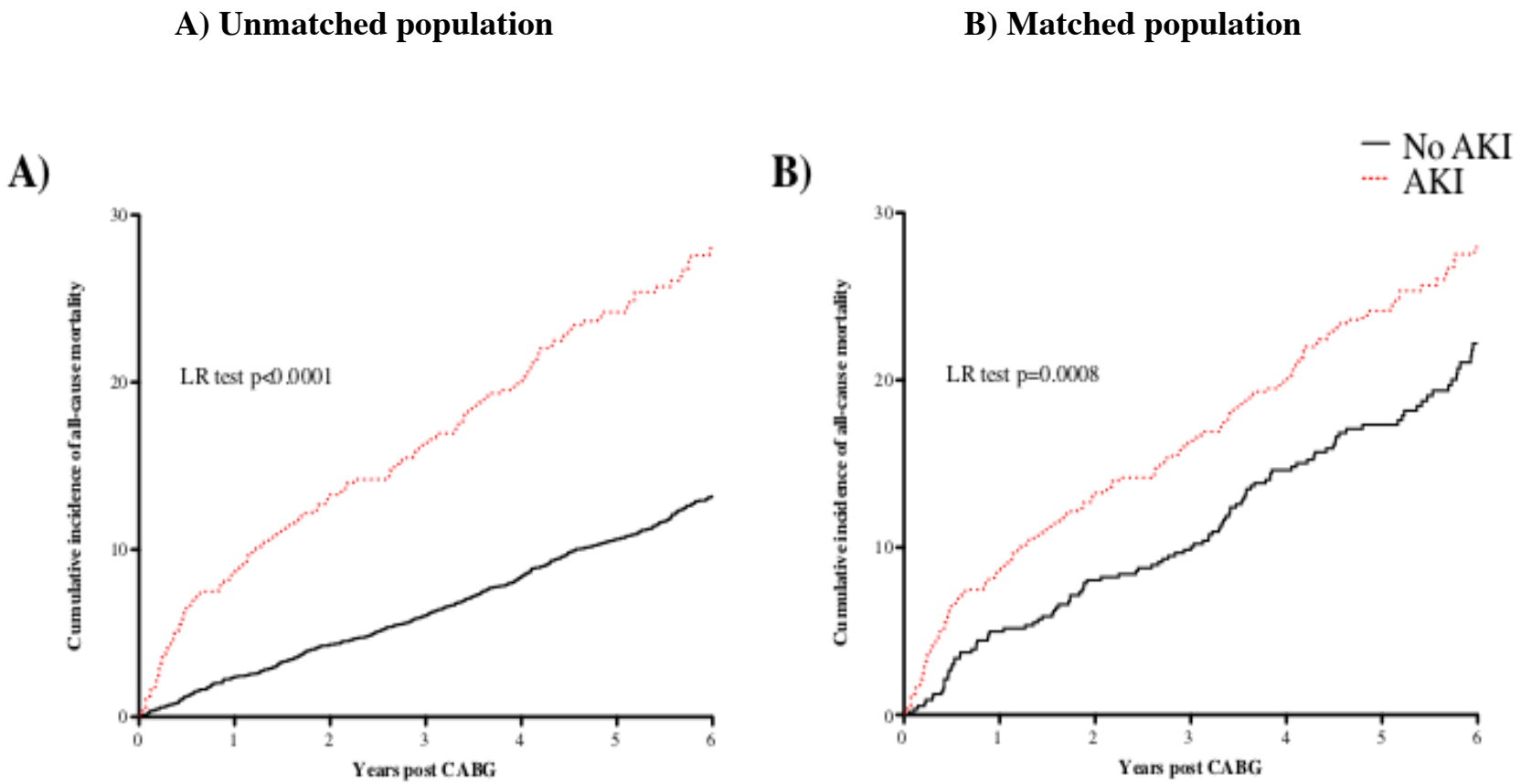
	Hazard Ratio	95% CI	P value
Age (per year)	1.06	1.05 - 1.07	<0.0001
Development of AKI	1.72	1.43 - 2.07	<0.0001
Diabetes Mellitus	1.33	1.13 - 1.56	<0.0001
COPD	1.61	1.30 - 1.99	<0.0001
Preoperative atrial fibrillation	1.47	1.06 - 2.06	0.023
LV ejection fraction <35%	2.04	1.63 - 2.54	<0.0001
3 vessel coronary disease	1.2	1.00 – 1.44	0.046
Baseline sCr	1.54	1.34 – 1.79	<0.0001
Procedural urgency	1.37	1.16 – 1.62	<0.0001

B) Matched population: Multivariate analysis

	Hazard Ratio	95% CI	P value
Age (per year)	1.05	1.04 – 1.07	<0.0001
Preoperative atrial fibrillation	1.68	1.08 – 2.63	0.023
Previous stroke	1.52	1.07 – 2.16	0.02
Procedural urgency	1.33	1.03 – 1.71	0.028
LV ejection fraction <35%	1.91	1.37 – 2.66	<0.0001
Development of AKI	1.52	1.20 – 1.94	0.001
Baseline sCr	1.40	1.18 – 1.68	<0.0001

COPD indicates chronic obstructive pulmonary disease; LV left ventricle; sCr serum creatinine.

Figure 6 Kaplan-Meier curves describing mid-term mortality after CABG in relation to the development of AKI



3.4 Discussion

In this large single centre cohort of patients undergoing first time CABG surgery who survived to hospital discharge the development of AKI was associated with an increase in all-cause mortality during mid-term follow-up. By 5 years after CABG surgery an absolute difference in mortality of 13.6% was evident between patients that sustained AKI and those that did not. Other than poor LV function, developing AKI was the most powerful independent predictor of death in this cohort.

The aim of this study was to investigate the association between peri-operative AKI, patient comorbidity and/or surgical complexity upon mid-term mortality following cardiac surgery. I was interested in establishing if AKI was an independent predictor of adverse outcome in this cohort. Consistent with my findings, numerous studies have reported that the development of AKI following cardiac surgery is associated with an increase in long-term mortality [113, 170, 171]. Furthermore, it has been clearly shown that patients with the highest burden of pre-operative comorbidity and those undergoing the most complex cardiac surgeries more commonly develop post-surgical AKI [98, 106, 107]. Importantly pre-surgical and surgical factors may confer a worse post surgical prognosis independent of the development of AKI and thus confound previous analyses.

As expected, in this cohort patients that developed AKI were older, had more comorbid disease, worse pre-operative renal function, and were more likely to undergo urgent surgery. Potentially any or all of these factors may affect subsequent mortality. However, when the effect of comorbidity and surgical complexity were controlled in a multivariable model the development of AKI remained a powerful independent predictor of mortality (HR 1.72). To further assess the association between AKI and post-operative mortality a propensity-score matching algorithm was used to match comorbidity and surgical complexity between patients that developed AKI and those that did not. This matching approach achieved two well-balanced groups for both comorbidities and surgical factors. After matching for comorbidity, the development of AKI still remained associated with a significant increase in 5-year mortality (24.2% vs 17.3%). Because no matching process can eliminate all differences between two groups of subjects, multivariable analysis was used to control for remaining differences in baseline comorbidity. After multivariable analyses, AKI remained an independent predictor of mid-term mortality risk in the propensity-matched cohort (HR, 1.52).

This is the first study to use a propensity-score matching approach to assess the association between post-operative AKI and mid-term mortality. Although propensity score matching is usually used to account for treatment selection bias in observational studies, it can also be used to compile patient cohorts with and without an exposure who are equivalent in all other baseline factors. Propensity-score matching was performed using AKI as the dependent variable without knowledge of mid-term mortality outcomes, just as investigators remain blinded during a randomized clinical trial. The propensity-score matching algorithm allowed patient and surgical factors associated with the development of AKI to be balanced between the AKI and the matched no-AKI control group. The mortality in the propensity score matched no AKI control group was higher than that seen in the unmatched population no AKI group highlighting the long-term prognostic importance of these AKI related factors incorporated into the propensity-score algorithm. Despite the use of these statistical techniques, a cause and effect relationship between post-operative AKI and mid-term mortality cannot be established with this observational data alone.

When interpreting these results, it is important to note that the 30-day morbidity and mortality rates for this surgical cohort appear low. This finding is explained by the choice to prospectively exclude patients that died in hospital post-operatively from subsequent analysis. A significant proportion of these patients developed AKI. These patients most likely represented a group with multi-organ failure rather than a primary renal pathology. Inclusion of these patients with multi-organ failure who died early may have confounded any subsequent analysis of the effect of AKI upon mid-term mortality.

AKI following cardiac surgery most likely results from a multifactorial renal insult that includes ischaemia-reperfusion injury, the systemic inflammatory response to CPB, peri-operative haemodynamic instability, micro-embolism and/or the peri-operative administration of nephrotoxins (such as NSAIDs, ACE inhibitors or iodinated contrast media) [172]. Why AKI is associated with long-term mortality is incompletely understood. In some patients the surgical procedure may result in irreversible renal injury and the development of CKD [96]. Post-operative sCr is a powerful predictor of long term mortality following cardiac surgery [173]. The requirement for post-operative dialysis is an extremely serious event associated with mortality rates as high as 60% [5]. However, AKI with complete resolution of renal function (measured by sCr) is also associated with an increase in long-term mortality, although it is suggested that a number of these patients may subsequently develop CKD [114]. As previously discussed the development of CKD is

strongly associated with the development and progression of cardiovascular disease, and is likely to be a driver of adverse cardiac outcomes in these patients. Currently there is little specific follow-up of patients that develop post operative AKI.

3.4.1 Study Strengths and Limitations

The main strength of this study is that it objectively assesses outcomes following CABG in a large contemporary cohort of consecutive patients. This means that our results are relevant to a broad patient population. Prospectively collected data upon short-term morbidity and mortality were collected in addition to the primary end point of all-cause mortality.

There are a number of limitations to this study worthy of note. Firstly, the primary end-point is all-cause mortality rather than cardiac death, and it is possible that other disease processes could have affected outcome. Second, as with all observational cohort studies, it was open to residual bias and unknown confounding factors. The propensity score-matching algorithm allowed the generation of comparable study groups, but our c-statistic for the algorithm suggests that there may be missing covariates that could affect mid-term mortality after CABG. The purpose of multivariable analysis of the propensity-matched cohort was to correct for any residual differences in baseline comorbidity. Third, I have no data upon post-operative urine output. Urine output in addition to sCr form the basis of the RIFLE classification system. It is possible that some patients that did not meet RIFLE sCr criteria would have been reclassified as having sustained AKI if urine output data was available. Fourth, the risk of AKI after cardiac surgery is increased when coronary angiography and surgery occur in close succession. I have no data on timing of coronary angiography in relation to cardiac surgery. Because all elective patients were discharged from the hospital after coronary angiography to wait for admission for cardiac surgery at a later date, it is likely that early cardiac surgery after coronary angiography only occurred in patients undergoing urgent cardiac surgery. Procedural urgency was incorporated within the propensity-matching model and, thus, was evenly balanced between the AKI and propensity-matched no AKI groups. Finally, patients that sustain AKI following cardiac surgery may be less likely to receive beneficial cardiovascular risk modification therapies prior to hospital discharge (most notably ACE inhibitors). I have no data upon patient's medical therapy at hospital discharge. If these therapies are not subsequently optimized during outpatient follow-up this may affect long-term outcome.

3.5 Conclusion

In this large single centre observational study the development of AKI following CABG surgery is independently associated with worse mid-term survival, both in the unmatched and propensity-score matched cohorts. The multivariable models and propensity score matching approaches suggest that this excess mortality following hospital discharge cannot be explained simply by pre-operative comorbidity or surgical complexity.

Chapter 4

4. A randomized controlled trial of remote ischaemic preconditioning in patients with chronic kidney disease for the prevention of myocardial and kidney injury after cardiac surgery.

Introduction

Perioperative complications of cardiac surgery including myocardial infarction (MI) and acute kidney injury (AKI) are associated with substantial morbidity and mortality. We assessed the impact of remote ischaemic preconditioning (RIPC) upon these complications in patients with chronic kidney disease (CKD).

Methods

86 adult patients with CKD (defined as eGFR < 60mL/min) undergoing coronary surgery with or without concomitant aortic valve replacement with cardiopulmonary bypass were randomized 1:1 to standard care with (n=43) or without (n=43) RIPC. RIPC consisted of three 5 min cycles of forearm ischaemia and reperfusion. The primary end points for this trial were two fold; the primary renal end point was development of AKI defined as a 50% post operative increase in sCr within 5 days of surgery. The primary cardiac end point was myocardial injury defined by 72 h troponin T area under the curve (72 h AUC cTnT). In addition, serum (NGAL, IL-18 and cystatin C) and urinary (NGAL, IL-18 and KIM-1) biomarkers of renal injury were also measured.

Results

Clinical and operative characteristics were similar between RIPC and control groups. 16 of 86 patients developed postoperative AKI. The incidence of AKI was similar in both groups

(RIPC 10/43 (23.3%) vs control 6/43 (14.0%); p=0.4065). There was no difference between the RIPC and control groups in the primary cardiac endpoint of 72 h AUC cTnT (RIPC 34686 vs control 31269 ng/L/72h; p=0.3668). Furthermore, there were no differences in any of the serum or urinary biomarkers of renal injury measured between the RIPC and control groups following surgery.

Conclusions

RIPC using forearm ischaemia confers no meaningful additional renal or myocardial protection in patients with CKD undergoing cardiac surgery.

4.1 Introduction

In recent years we have seen a change in the profile of preoperative medical comorbidity of patients accepted for cardiac surgery [1]. It is now commonplace for patients with conditions such as diabetes, peripheral vascular disease or left ventricular dysfunction to be offered surgery, when previously they would not have been considered 'fit' enough for these major operations. Unsurprisingly, these 'higher-risk' patients are more susceptible to peri-operative complications including MI, congestive cardiac failure, AKI and/or stroke. They are also more likely to require high-level post-operative critical care support, and have extended post-operative hospital stays.

As I have shown in Chapter 2 patients with advanced CKD represent a group at especially elevated risk of adverse events following cardiac surgery. A particular peri-operative problem for patients with CKD is the development of AKI. Chapter 3 demonstrates that AKI is a harbinger of poor prognosis after cardiac surgery independent of baseline pre-operative comorbidity. Currently there are no effective therapies to prevent or treat AKI in patients undergoing cardiac surgery. This chapter details a randomized control trial investigating whether forearm remote ischaemic preconditioning (RIPC) is effective in reducing the incidence of peri-operative AKI and myocardial injury in patients with CKD undergoing cardiac surgery.

4.1.1 Ischaemia reperfusion injury

The most common clinical example of ischaemia reperfusion injury (IRI) is acute MI. Interruption of coronary blood flow results in ischaemic myocellular injury. Early

reperfusion of ischaemic myocardium can potentially salvage viable myocardium and limit infarct size. However, reperfusion can itself paradoxically induce further injury and cell death; the so called 'double edge sword' of reperfusion [174]. This lethal reperfusion injury attenuates the benefit of reperfusion strategies such as PCI or thrombolysis for MI.

Although not yet fully understood the key cellular mechanisms of IRI are thought to include intracellular calcium overload and redistribution, overproduction of reactive oxygen species (ROS), rapid fluxes in intracellular pH and a local and systemic inflammatory reaction [175]. These processes culminate in cell death through the opening of the mitochondrial permeability transition pore (mPTP) [175]. Usually the mitochondrial membrane is impermeable to ions and metabolites. During IRI a combination of intracellular events result in the mPTP becoming permeable to molecules of 1500kDa or smaller. Opening of the mPTP collapses the mitochondrial membrane potential and uncouples oxidative phosphorylation, resulting in ATP depletion and eventual cell death{Kharbanda, [175, 175-177].

In cardiac surgery IRI results from aortic cross-clamping. Aortic cross-clamping is a fundamental component of modern cardiac surgery that allows a blood-free operative field for the surgeon. However clamping and unclamping of the ascending aorta causes episodes of IRI that may result in peri-operative myocardial and kidney injury. To mitigate the insult of aortic cross clamping, myocardial protection strategies including electrochemical cardiac arrest induced by perfusing the heart with cardioplegic solution (either via the aortic root or coronary sinus) or hypothermic intermittent cross-clamp fibrillation have evolved. Despite these modern myocardial protection techniques 'high-risk' cardiac surgical patients still sustain significant IRI with subsequent clinical end-organ dysfunction.

Novel strategies to reduce IRI and enhance clinical outcomes in 'high-risk' cardiac surgical patients are urgently needed. To date clinical research to modulate IRI has been in three main areas,

1. Induction of endogenous protection via local and remote ischaemic conditioning.
2. Pharmacological induction of endogenous protection.
3. Pharmacological therapy targeting components of the IRI cascade [178].

The technique that has shown most promise in modifying IRI to date has been ischaemic preconditioning.

4.1.2 Ischaemic preconditioning

The heart possesses endogenous mechanisms capable of protecting against IRI. Ischaemic preconditioning is the term given to the finding that brief episodes of non-lethal ischaemia and reperfusion applied directly to an organ, can protect the same organ from a subsequent episode of sustained lethal IRI. This phenomenon was first described in 1986 using an animal model of MI [179]. Four 5 min cycles of myocardial ischaemia and reperfusion rendered the myocardium resistant to a subsequent prolonged (40 min) episode of ischaemia and reperfusion. The resultant MI in conditioned hearts was reduced to 25% of the size of that observed in the non-conditioned control hearts. Many of the ensuing studies into ischaemic preconditioning have focused upon the heart, but more recently ischaemic preconditioning has been demonstrated in a variety of other organs including the kidney [180], liver [181], and brain [182]. However, it remains possible that each organ has a 'preconditioning threshold', such that a preconditioning stimulus sufficient to induce the protection from an ischaemic insult in one organ may not prove protective in all organs.

The mechanisms of ischaemic preconditioning are as yet not fully elucidated, although many of the signal transduction pathways involved have been deciphered. For example, ischaemic precondition activates the cyclic guanosine monophosphate/cGMP-dependent protein kinase (cGMP/PKG) pathway [183], reperfusion injury salvage kinase (RISK) pathway [184] and the survivor activating factor enhancement (SAFE) pathway [185]. These pathways terminate at the mitochondria resulting in the opening of mitochondrial KATP channels and the generation of mitochondrial ROS, which may mediate cellular protection by inhibiting the opening of the mPTP [186].

Animal Studies suggest there are two distinct periods of organ protection induced by ischaemic preconditioning. The first is early, named acute or classical ischaemic preconditioning that is immediate but transient, disappearing approximately 3 h after the preconditioning stimulus [175, 179]. The second window of protection is known as delayed or late ischaemic preconditioning and appears 12 to 24 h after the conditioning stimulus and lasts for up 3 days [187].

Despite promising preclinical work ischaemic preconditioning has not been translated into a clinical therapy. Translation has been limited by the need for the conditioning stimulus to be applied before a sustained episode of lethal ischemia, and also that the conditioning stimulus must be applied directly to an organ. Consequently the settings in which ischaemic

preconditioning may be beneficial are limited. In cardiac surgery both of these prerequisites can be met. IRI associated with aortic cross-clamping is predictable and direct access to the heart is possible. The first human trial of ischaemic preconditioning in cardiac surgery was conducted in 1993 [188]. The preconditioning stimulus was two cycles of 3 min of aortic cross-clamping with 2 min of reperfusion prior to cardiac arrest induced with intermittent cross-clamp fibrillation. Patients that received preconditioning had higher myocardial ATP levels in ventricular biopsies and lower levels of serum troponin T (cTnT) than patients in the control group. More than 20 studies of ischaemic preconditioning in cardiac surgery have now been completed. A meta-analysis (22 studies collectively involving 933 patients) of ischaemic preconditioning published in 2008 concluded that ischaemic preconditioning was associated with a reduced frequency of ventricular arrhythmias, reduced post-operative inotrope requirement and shorter stay on the intensive care unit [189]. Notwithstanding these studies, ischaemic preconditioning has not become commonplace in cardiac surgery. Potential reasons for this lack of uptake include,

1. A reluctance of surgeons to apply an invasive conditioning stimulus that prolongs the duration of the surgery.
2. The finding that volatile anaesthetic agents also confer preconditioning effects.
3. That the very patients that ischaemic preconditioning may benefit most (the elderly or those with significant comorbidity) may be resistant to preconditioning
4. That aortic cross-clamping predisposes to atheroembolic complications [190].

4.1.3 Remote ischaemic preconditioning

Remote ischaemic preconditioning (RIPC) circumvents the need to apply a direct conditioning stimulus to the heart, which is both inconvenient and potentially harmful. RIPC is the term used to describe the finding that the classic ischaemic-preconditioning stimulus of brief episodes of non-lethal ischaemia and reperfusion is still effective when applied to a remote organ or tissue. RIPC was first demonstrated in a canine model of MI [191]. The application of four 5-min cycles of ischaemia and reperfusion to the circumflex coronary artery reduced the size of a subsequent myocardial infarction generated by 4.5-h of occlusion of the left anterior descending artery by 70% compared with control. Ensuing studies have demonstrated that cardiac RIPC can be achieved by applying ischaemia and reperfusion to remote organs such as the kidney, liver or intestine [192] but most clinically relevant, it can be generated by preconditioning the upper or lower limb [193].

The precise mechanism of RIPC is currently unclear although several hypotheses to explain this phenomenon exist. For example, it is proposed that local production of an endogenous substance generated in the ischaemic organ such as adenosine, bradykinin or calcitonin gene-related peptide (CGRP) may potentially activate neural pathways that terminate at the heart mediating cardioprotection [186]. Supporting this theory, autonomic ganglionic blockade in a rat MI model abolished cardioprotection induced by RIPC achieved by mesenteric artery occlusion but had no effect on myocardial IPC [192]. An alternative hypothesis is that a locally produced humoral factor such as adenosine, bradykinin, opioids, CGRP, endocannabinoids, or Angiotensin I in the ischaemic organ enters the blood stream and directly activates a cellular receptor in the myocardium [186]. Evidence for the involvement of such a factor is supported by the observation that in a rabbit MI model, cardioprotection can be transferred by the transfusion of serum from a rabbit that has undergone ischemic preconditioning to one which has not [194]. However these cardioprotective signals reach the heart, once there, they are thought to activate intracellular signal transduction mechanisms similar to those that participate in IPC.

The first demonstration of RIPC in humans was in 2002 [195]. A RIPC protocol consisting of 3 cycles of 5 min of forearm ischaemia (generated using a blood pressure cuff inflated to 200 mmHg on the upper arm) with 5 min of intervening reperfusion was applied to healthy volunteers. Subsequently contralateral forearm endothelial IRI was induced (using a blood pressure cuff inflated to 200 mmHg on the upper arm for 20 min followed by reperfusion). Endothelial IRI was assessed using strain-gauge plethysmography to measure forearm blood flow response to the endothelium-dependent vasodilator dilator acetylcholine (ACh). In the control group the expected increase in blood flow following ACh was significantly attenuated (due to endothelial IRI). Forearm blood flow increased as expected in the RIPC group suggesting that RIPC induced with intermittent forearm ischaemia conferred a protective effect against endothelial IRI. This less invasive technique has led to a resurgence of interest in translating ischaemic preconditioning into a useful clinical therapy. The flexibility of using intermittent limb ischaemia as a conditioning stimulus enables RIPC to be applied in a wide variety of clinical settings, for example MI, cardiac surgery, cardiac arrest, vascular surgery or organ transplantation.

4.1.4 Remote ischaemic preconditioning as cardioprotection during cardiac surgery

Despite modern cardioprotective strategies patients undergoing cardiac surgery may experience substantial peri-operative myocardial injury. This is due to IRI induced during

the operation by aortic-cross clamping, manual handing of the heart, microembolization of atheromatous debris and systemic inflammation due to CPB[196]. Surrogate measures of peri-operative myocardial injury, including serum cardiac enzymes (either creatine kinase or cTnT) or area of myocardial necrosis measured using late gadolinium enhancement cardiac MRI (LGE-CMR), have been associated with worse post-surgical outcomes [196].

The first study of RIPC induced by transient limb ischaemia in cardiac surgery was a small pilot study of only eight patients undergoing CABG[197]. Preconditioning was achieved using a tourniquet placed on the upper arm to render the forearm ischaemic. The study was negative, with no difference in serum CK-MB 5 min after de-clamping of the aorta. Interpretation of this study is difficult as it is severely underpowered with only four patients in the control and RIPC arms and the preconditioning stimulus of two cycles of 3 min ischaemia with 2 min reperfusion may have been inadequate to trigger endogenous myocardial protection. The failure to examine serum cardiac enzymes beyond 5 min post aortic de-clamping is also a signification limitation of this study.

The first positive study of RIPC in cardiac surgery was a pilot study conducted in 37 children undergoing surgery to correct congenital heart defects [198]. The preconditioning stimulus was 4 cycles of 5 min leg ischaemia with 5 min reperfusion. Children that received RIPC had less perioperative myocardial injury (measured using 24 h AUC troponin I (cTnI)), lower inotrope usage and improved lung function when compared with children who received the control treatment of a non-inflated blood pressure cuff applied to the thigh pre-operatively.

In 2007 the first successful trial of RIPC in adult patients undergoing CABG was published [199]. In this trial, 57 elective patients undergoing CABG were randomized to receive RIPC (induced using 3 cycles of 5-min forearm ischaemia with 5 min reperfusion using a blood pressure cuff on the upper arm inflated to 200mmHg) or control (a deflated blood pressure cuff placed on the upper arm for 30 minutes) in the anaesthetic room prior to surgery. Perioperative myocardial injury (measured using 72 h AUC cTnT) was 43% less patients that received RIPC compared with the control group. Following this landmark clinical study the interest in RIPC to reduce peri-operative myocardial injury has grown rapidly. There are now numerous clinical studies of RIPC in different cardiac surgical settings (**Table 10**)

Table 10 Clinical studies of remote ischaemic preconditioning in patients undergoing cardiac surgery (adapted from [196])

Study	RIPC protocol (min), (site)	Patients	Outcome
Günaydin et al [197]. (2000)	3×3 (leg)	8 adults undergoing CABG surgery±valvoplasty	No change in CK-MB 5min after aortic unclamping
Cheung et al [198]. (2006)	4x5 (leg)	37 children (aged 1–2 years) undergoing corrective cardiac surgery for congenital heart disease	Decreased 24 h AUC troponin I, Decreased inotrope score, Decreased airway pressures
Hausenloy et al [199] (2007)	3x5 (arm)	57 adults undergoing CABG surgery±valvoplasty	Decreased 72 hour trop T AUC (by 43%)
Venugopal et al [200]. (2009)	3x5 (arm)	45 adults undergoing CABG surgery±valvoplasty	Decreased 72 hour trop T AUC (by 42%)
Wenwu et al [201]. (2010)	3x5 (arm)	60 children (aged <7 years) undergoing corrective cardiac surgery for congenital heart disease	Decreased 2h, 4h, 12h, 24h CK-MB and troponin T
Thielmann et al [202]. (2010)	3x5 (arm)	53 adults undergoing CABG surgery	Decreased 48h AUC troponin I (by 35%)
Rahman et al [146]. (2010)	3x5 (arm)	162 adults undergoing elective or urgent CABG surgery	No difference in 48h troponin T or LVEF
Wagner et al [203]. (2010)	3x5 (arm)	101 adults undergoing CABG surgery±valvoplasty	Decreased 8h troponin I (by 27%)
Ali et al [204]. (2010)	3x5 (arm)	100 adults undergoing elective CABG surgery	Decreased 6h, 24h, 48h CK-MB and troponin T
Xie et al [205]. (2012)	3x5 (arm)	73 adults undergoing valvular surgery	Decreased 72hr AUC troponin T (by 44%)
Young et al [206]. (2012)	3x5 (arm)	96 adults undergoing "high risk ' cardiac surgical procedures	Increased 6hr and 12 hr troponin T in RIPC group

However, these studies are not uniformly positive. For example the largest study of RIPC in cardiac surgery to date (162 patients undergoing either elective or urgent CABG) failed to show any effect upon perioperative myocardial injury (measured using 48 h AUC troponin release), inotrope requirements, renal or lung injury following CABG[146]. Potential explanations for the discrepant study results include:

Concomitant medications: Volatile anaesthetic gases such as isoflurane and sevoflurane can decrease infarct size in animal models [207]. Furthermore, both propofol and intravenous nitrates have been reported to protect the heart against IRI during cardiac surgery [208]. Therefore, it is conceivable that specific anaesthetic regimes may either augment or ameliorate the effects of RIPC.

Comorbidity: Potential benefits of ischaemic preconditioning may be reduced by a number of clinical conditions that coexist in patients with coronary disease [209]. For example, diabetes affects potassium ATP channels, thought to be important in the mechanism of preconditioning, and so may reduce the protection conferred by preconditioning [210]. Studies also suggest that hyperlipidemia, hypertension and age may reduce the effect of preconditioning [211]. MI may induce a conditioning stimulus. Thus patients undergoing early surgery following MI will gain little additional benefit from another preconditioning stimulus immediately before surgery [196]. Ironically this means that patients with the highest burden of comorbidity undergoing urgent high-risk surgery are the very group who may not respond to this intervention.

Surgical characteristics: whether aortic cross-clamp time or duration of cardiopulmonary bypass affects the efficacy of ischaemic preconditioning is unknown. It has also been suggested that cardiopulmonary bypass itself may be a preconditioning stimulus [212].

In order to definitively assess the effect of RIPC upon perioperative myocardial injury and clinical outcomes after cardiac surgery two large multicentre clinical trials are currently ongoing.

4.1.5 Remote ischaemic preconditioning to protect the kidney during cardiac surgery

IRI is an important component in the mechanism of AKI following cardiac surgery. Consequently there is potential for RIPC to reduce its incidence. RIPC has been reported to ameliorate renal injury after abdominal aortic aneurysm (AAA) surgery [144], but to date there is limited data investigating the reno-protective effect of RIPC in cardiac surgery (**Table 11**).

The first report of RIPC as a reno-protective strategy in cardiac surgery was a retrospective secondary analysis of renal outcomes drawn from 2 randomized trials of RIPC used for myocardial protection [145]. RIPC using three 5 min cycles of ischaemia and reperfusion of the forearm was associated with a 14.5% reduction in the incidence of AKI (defined by AKIN criteria as a 26.4 μmol/L increase in post-operative sCr). Important criticisms of this data are that the intervention and control groups are imbalanced in terms of pre-operative comorbidity, and that patients with either diabetes or CKD were excluded from the trial. Furthermore, as it is a retrospective secondary analysis drawing any firm conclusions is difficult.

Table 11 Clinical studies of remote ischaemic preconditioning as renoprotection in patients undergoing cardiac surgery

Study	RIPC protocol (min)	Patients	Outcome
Venugopal et al [145]. (2010)	3x5 (arm)	78 adults undergoing elective CABG	RIPC decreased the incidence of AKI (by 14.5%).
Rahman et al[146]. (2010)	3x5 (arm)	162 adults undergoing elective or urgent CABG surgery	Day 4 SCr levels and incidence of AKI were not different between groups.
Choi et al [147]. (2011)	3x10 (leg)	76 adults undergoing complex valvular surgery	No difference in post-operative incidence of AKI or levels of renal biomarkers (serum NGAL or cystatin C).
Zimmerman et al [148]. (2011)	3x5 (leg)	120 adult undergoing cardiac surgery with cardiopulmonary bypass	RIPC decreased the incidence of AKI (by 27%).
Pederson et al [213]. (2012)	4x5 (leg)	115 children undergoing corrective cardiac surgery for congenital heart disease	No difference in post-operative incidence of AKI or levels of renal biomarkers (serum/urine NGAL or cystatin C).
Young et al [206]. (2012)	3x5 (arm)	96 adults undergoing ‘high risk ‘ cardiac surgical procedures	No difference in post-operative incidence of AKI

The first prospective study designed to investigate the effects of RIPC upon renal function post cardiac surgery was published in 2011 [147]. This study enrolled patients undergoing complex cardiac surgery that predisposed to post-operative AKI. RIPC induced by three 10-min cycles of leg ischaemia did not affect the incidence of AKI (defined by AKIN criteria) or renal biomarker release profiles (serum NGAL and serum cystatin C) post cardiac surgery. Although anaesthetic regimes were standardized the nature of cardiac procedures were quite different between the RIPC and control groups. Potentially the heterogeneity in surgical procedures may have affected the results of this study. Later the same year the first positive reno-protective study of RIPC in cardiac surgery was published [148]. This trial randomised 120 patients undergoing elective cardiac surgery to receive RIPC (induced using 3 cycles of 5 min of leg ischaemia with 5 min of reperfusion) or a control group. There was an absolute reduction in the incidence of AKI (defined by AKIN criteria) of 27% in the patients that received RIPC compared with the control group. The single greatest risk factor for developing AKI following cardiac surgery is pre-existing kidney disease. Although patients with CKD were not excluded from this study, only 16% of enrolled patients had an eGFR < 60 mL/min. Whether patients with CKD respond to renal RIPC remains unknown. Animal models suggest that uraemia alone does not reduce the effects of cardiac RIPC [214].

4.1.6 Rationale for current study

Patients with CKD (defined as eGFR < 60mL/min) make up approximately 30% cardiac surgical patients [215]. These patients are at high risk of adverse events post-operatively; 30-day mortality rates are markedly increased in these patients and CKD is the major risk factor for the development of post-operative AKI [3]. RIPC has shown promise as both a cardioprotective and reno-protective measure in patients undergoing cardiac surgery. Patients with CKD may accrue significant benefit from RIPC given the increased peri-operative risk they face. Whether RIPC is efficacious in this patient population is unknown. We have undertaken a randomized controlled trial to assess the effect of RIPC upon perioperative myocardial and renal injury in patients with CKD undergoing cardiac surgery.

4.2 Methods

4.2.1 Trial overview

This was a single-center, single-blinded, prospective, randomized, placebo-controlled trial of RIPC in patients with CKD undergoing first time CABG with or without concomitant aortic valve replacement (AVR) on CPB. The trial was approved by the local research ethics committee (MREC No. 10/H0703/92), and was registered with the Barts Health research and

development department (R&D No. 007424). Written informed consent was obtained from each study subject.

4.2.2 Study Hypothesis

The hypothesis was that RIPC would reduce perioperative myocardial injury (assessed using 72 h AUC serum troponin T level) and the incidence of AKI (defined as a 50% post-operative increase in sCr within 5 days of cardiac surgery) compared to standard care in patients with CKD undergoing cardiac surgery.

4.2.3 Patients and Setting

We enrolled patients with CKD (eGFR <60 mL/min) undergoing either elective or urgent (post acute coronary syndrome) surgery at Barts Health NHS Trust between February 2011 and April 2012. GFR was estimated using the Modification of Diet in Renal Disease equation [7] on two separate occasions pre-operatively; for elective patients this was in a pre-admission clinic approximately 1 month before surgery and also on the day before the operation. For patients undergoing urgent surgery GFR was estimated at hospital admission and again on the day before the operation. All eligible patients meeting trial inclusion criteria, with no exclusion criteria (**Table 12**) were approached for trial enrollment.

Table 12 Study inclusion and exclusion criteria

Inclusion Criteria	1.	Planned to undergo elective or urgent CABG with or without concomitant AVR
	2.	CKD defined by an eGFR < 60mL/min on 2 occasions prior to cardiac surgery
	3.	Planned use of CPB
	4.	Age > 18 and < 85 years
	5.	Able to give informed consent
Exclusion Criteria	1.	Myocardial infarction within 1 week of surgery
	2.	Planned 'off-pump' cardiac surgery
	3.	Premorbid ESRD or previous renal transplant
	4.	Preoperative AKI: defined as an increase in sCr > 50% from either pre-admission or admission value to value obtained on the day before surgery
	5.	Coronary angiography within 1 week of surgery
	6.	Enrolled in a conflicting study
	7.	Pregnancy
	8.	Unable to give informed consent

4.2.4 Sample size

This was a pilot study: At study conception there was no published data describing the effect of RIPC upon the incidence AKI following cardiac surgery. However, published data suggested that RIPC may reduce myocardial injury following CABG by 43% (measured by 72 h AUC cTnT) [199]. Patients with CKD have a high prevalence of co-morbid conditions (such as diabetes mellitus and hypertension) and may have a reduced response of RIPC. We assumed a less dramatic response to RIPC in our cohort of a 25% reduction in myocardial injury. To detect a difference of at least 25% between the two groups, with power of 95% and confidence intervals of 95% we needed to recruit at least recruit 41 pairs of patients.

4.2.5 Randomisation

Patients were stratified by type of surgery (CABG or CABG with AVR) and presence of diabetes mellitus and were then randomized within strata using a computer generated randomization tool to remote ischaemic preconditioning or control group in a 1:1 ratio in randomly sequenced blocks of 4 or 6, which were then concealed using numbered sealed envelopes. Patients, anesthetists, surgeons, and critical care teams were all blinded to study group allocation, although investigators were not blinded.

4.2.6 Study protocol (Figure 7)

For patients randomized to receive RIPC a 9cm blood pressure cuff was placed upon the upper arm prior to anaesthetic induction. After induction, the cuff was inflated 3 times to a pressure of 200mmHg, or a pressure of 50mmHg greater than systolic blood pressure, for 5 min with an intervening 5 min of reperfusion between cuff inflations. RIPC was conducted while patient monitoring, intravascular catheters, and an indwelling bladder catheter were being placed in order not to affect the time from anaesthetic induction to CPB. Control patients had an identical deflated blood pressure cuff placed on the upper arm (but not inflated) for 30 min. All patients otherwise received standard intraoperative and perioperative care at the discretion of the cardiac anesthetic teams.

Cardiac surgery was undertaken using standard non-pulsatile CPB with a membrane oxygenator and cardiotomy suction. The coronary bypass grafts, either left internal mammary artery or saphenous venous grafts were constructed whilst on CPB with each anastomosis to the coronary arteries completed with the use of either intermittent cross-clamp fibrillation or cardioplegia. Once all of the grafts were constructed CPB was discontinued. Post-operative care was at the discretion of the critical care team.

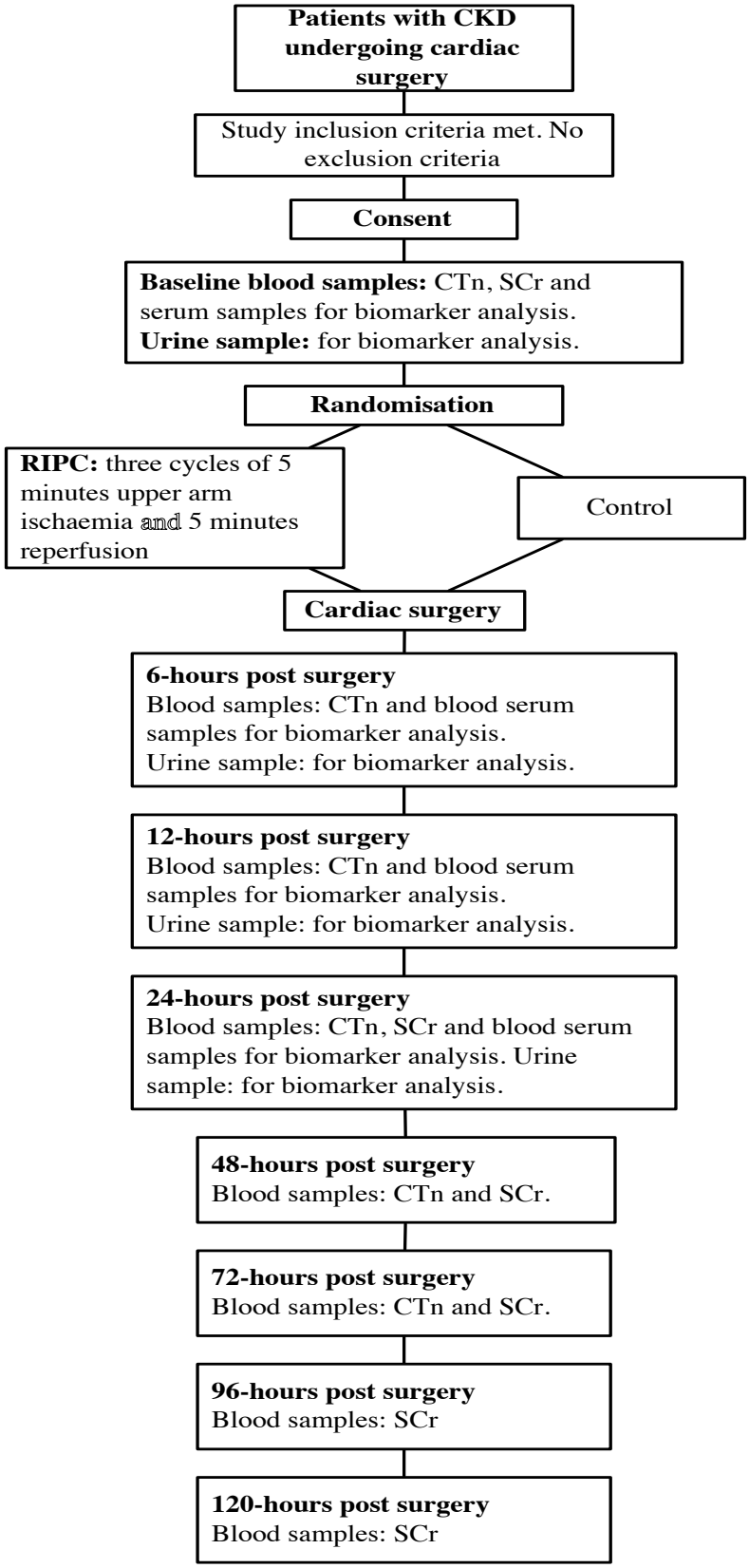


Figure 7 Study protocol

4.2.7 Data collection

Detailed demographic and clinical information was recorded from the hospital clinical records at trial enrollment. This information included age, sex, ethnicity, clinical presentation (either elective or urgent), BMI, NYHA class, history of previous MI, PCI, diabetes mellitus, diabetic treatment status, hypertension, hypercholesterolaemia, PVD, stroke, COPD, pre-operative renal status (including sCr, eGFR, and urinary protein creatinine ratio), anatomical severity of CAD, pre-operative left ventricular function (obtained by either echocardiography, contrast ventriculography or CMR imaging) and logistic EuroScore [157].We also recorded the date of any recent MI and cardiac catheterization along with a detailed pre-operative drug history.

Procedural information recorded included the method of cardioprotection employed (cardioplegia or intermittent cross-clamp fibrillation), cross clamp time, perfusion time, use of internal mammary arterial (IMA) grafts, number of coronary bypass grafts constructed and types of volatile anesthetic used.

Post operatively urine output was recorded hourly; hemodynamics and inotrope scores recorded every 3 h for the first 24 h. The volume of crystalloid, colloid and blood products administered over the first 24 h were also recorded. The inotrope score was calculated from the dose of the individual inotropes used as follows [198],

Inotrope score = Dosages (in μg/kg/min) of

1. Dopamine + Dobutamine +
2. [(Adrenaline + Noradrenaline + Isoproterenol + Isoproterenol) x 100] +
3. [Enoximone x 15]

Postoperative complications (including MI, arrhythmia requiring treatment and/or congestive cardiac failure), length of intensive care stay, hospital stay and 30-day mortality were also recorded. Perioperative myocardial infarction was defined by the presence of new left bundle-branch block or new Q waves of 2 mm in depth in 2 contiguous leads by postoperative day 3.

4.2.8 Biochemistry Samples

Blood samples for measurement of troponin T were taken before surgery and at 6, 12, 24, 48, and 72 h after surgery. cTnT concentrations were measured using the commercially available Elecsys electrochemiluminescence immunoassay (ECLIA) troponin T high sensitive assay kit. The coefficient of variation for intra-assay variation was 4.87% in our laboratory.

Blood samples for sCr were drawn less than 24 h before surgery and then daily for 5 days postoperatively unless the patient was discharged within this period. sCr was measured using a buffered kinetic Jaffe reaction on an automated clinical chemistry analyser (Roche Modular Analytics, Core unit and Control unit). The coefficient of variation for intra-assay variation for creatinine concentration was 3.75% in our laboratory.

Blood samples were collected for measurement of serum neutrophil gelatinase–associated lipocalin (NGAL), serum cystatin C (CyC), and serum interleukin-18 (IL-18) before surgery and at 6, 12 and 24 hours post-operatively. Serum was separated from blood by centrifugation at 1300 rpm for 15 minutes and stored at -70 ºC until assay. Urine samples were collected for measurement of urine NGAL, urine IL-18, urine kidney injury molecule-1 (KIM-1) and urine creatinine before surgery and at 6, 12 and 24 hours post-operatively. Urine samples were also centrifuged and stored at -70 ºC until assay.

Serum and urine NGAL levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (Human Lipocalin-2/NGAL Immunoassay; R&D Systems, Europe Ltd., Abingdon, U.K.) according to manufacturer instructions (see appendix 1). The coefficient of variation for intra-assay variation for NGAL concentration was 7.2% in our laboratory.

Serum and urine IL-18 levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (Human IL-18 BPa Immunoassay; R&D Systems, Europe Ltd., Abingdon, U.K.) according to manufacturer instructions (see appendix 1). The coefficient of variation for intra-assay variation for IL-18 concentration was 11.9% in our laboratory and corresponds to that reported by manufacturer.

Serum CyC level was detected by using a commercially available enzyme-linked immunosorbent assay kit (Human Cystatin C Immunoassay; R&D Systems, Europe Ltd., Abingdon, U.K.) according to manufacturer instructions (see appendix 1). The coefficient of variation for intra-assay variation for Cystatin C concentration was 5.1% in our laboratory.

Urinary KIM-1 level was detected by using a commercially available enzyme-linked immunosorbent assay kit (TIM-1/KIM-1/HAVCR Immunoassay; R&D Systems, Europe Ltd., Abingdon, U.K.) according to manufacturer instructions (see appendix 1). The coefficient of variation for intra-assay variation for KIM-1 concentration was 4.8% in our laboratory.

Urinary creatinine was detected by using a commercially available fluorometric assay (Creatinine Assay; Abcam, Cambridge, UK) (see appendix 1).

To adjust urinary biomarker concentrations to urinary creatinine concentration we calculated a ratio of urinary biomarker/urinary creatinine by dividing urinary biomarker concentration (ng/mL) by the urinary creatinine concentration (mg/mL).

4.2.9 End Points

The primary end points for this trial were two fold; the primary renal end point was AKI defined in accordance with RIFLE criteria as any post-operative increase in the sCr of greater than 50% from the preoperative value within 5 days of surgery. The primary cardiac end point was myocardial injury defined by 72 h AUC cTnT.

Secondary renal end points included,

- **Percentage change in sCr:** pre-operative baseline to post-operative peak (Δ SCr).
- **AKI defined by RIFLE class:** post-operative increases in sCr of 50 -100% were categorized as RIFLE R, increases in sCr of 100-200% were categorized as RIFLE I and increases in sCr > 200% were categorized as RIFLE F.
- **Duration of AKI:** patients with AKI were categorized as AKI duration for 1 to 2, 3 to 4, or > 5 days. For this sCr had to be >50% above the pre-operative value on each of the consecutive days.

- **Renal Biomarker end-points:** the effect of RIPC on serum and urine NGAL, serum and urine IL-18, serum CyC and urinary KIM-1 levels at 6h, 12 h and 24 h.

Secondary cardiac end points included,

- 6 hour cTnT.
- Peak cTnT.
- Incidence of post-operative MI.
- Occurrence of post-operative atrial fibrillation.

The effect of RIPC upon the need for dialysis during the index hospitalization, inotrope score, duration of mechanical ventilation, length of intensive care unit stay, length of hospital stay and 30 day mortality was also assessed

The primary cardiac and renal end points were also assessed in the following pre-specified sub-groups,

1. Non-diabetic patients
2. Patients undergoing elective surgery
3. Patients undergoing surgery with cardioplegia

4.2.10 Statistical analysis

All data were analysed according to the intention-to-treat principle. Normality of distribution of continuous data was assessed using the Shapiro-Wilks test. Normally distributed continuous data are presented as mean (±SD); whereas skewed continuous data are presented as median (interquartile range [IQR]). Normally distributed data were compared with an unpaired Student's t-test and non-normally distributed data were analysed non-parametrically (Mann-Whitney U test). Categorical data are summarized using absolute values (percentage). Categorical data were compared using the Pearson chi-square test or Fishers exact test where appropriate. All tests were two-tailed; statistical significance was indicated by a p-value <0.05. Univariate analyses and figure preparation was undertaken using GraphPad Prism 5 for MacOS (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com).

4.3 Results

One hundred and one patients with CKD underwent CABG with or without AVR within the study period. Eighty-six of these patients were recruited to the study (**Figure 8**).

Participant demographics, clinical characteristics and operative details were similar between the randomised groups. The demographic and clinical characteristics are described in **Table 13**. The operative characteristics are described in **Table 14**.

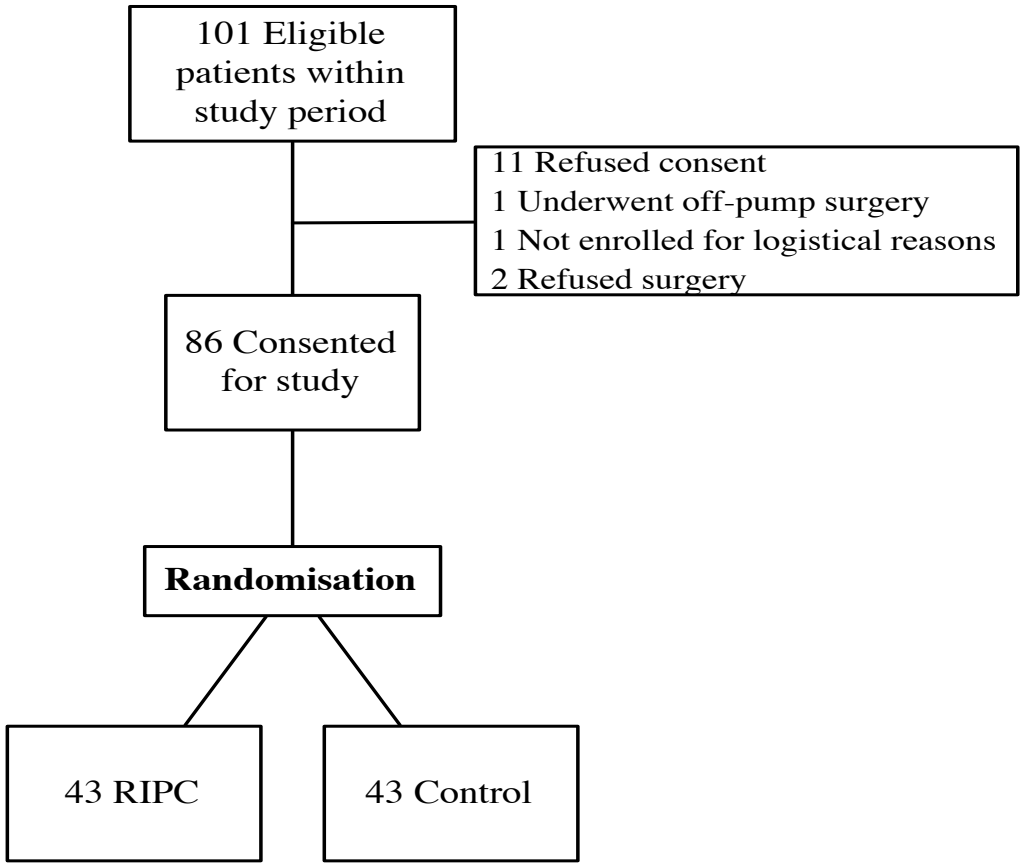


Figure 8. Trial flowchart. Enrolled patients are stratified by scheduled surgery and presence of diabetes mellitus then randomized to study groups.

Table 13 Baseline demographics and clinical characteristics of the study patients stratified by study group

Variable		RIPC (43 patients)	Control (43 patients)	p value
Demographics				
Age years±SD		68.7±11.0	72.8±8.4	0.107
BMI kg/m^2±SD		28.2±4.7	27.4±4.7	0.432
Gender (male:female)		33:10	36:7	0.417
Ethnicity				0.897
	Caucasian n,%	28 (65.1)	29 (67.4)	
	Afro-Caribbean n,%	3 (7.0)	2 (4.7)	
	South Asian n,%	11 (25.6)	10 (23.3)	
Pre-operative Symptom status				
NYHA class > 3 n,%		18 (41.8)	11 (25.6)	0.110
Clinical characteristics				
Diabetes Mellitus (DM)				0.630
	Non-insulin treated DM n,%	14 (32.6)	18 (41.9)	
	Insulin treated DM n,%	13 (30.2)	10 (23.3)	
Hypertension n,%		34 (79.1)	37 (86.0)	0.394
Hypercholesterolaemia n,%		37 (86.0)	30 (69.5)	0.069
Previous MI n,%		25 (58.1)	20 (46.5)	0.280
Previous stroke or TIA n,%		9 (20.9)	6 (14.0)	0.394
Peripheral vascular disease n,%		15 (34.9)	14 (32.6)	0.820
COPD n,%		5 (11.6)	4 (9.3)	0.725
Pre-operative Renal Function				
Median eGFR mL/min (IQR)		51 (42 to 54)	51 (43 to 55)	0.952
Median sCr μmol/L IQR		121 (111 to 143)	121 (113 to 143)	0.746
CKD stage				0.923
	Stage 3a n,%	29 (67.4)	28 (65.1)	
	Stage 3b n,%	11 (25.6)	11 (25.6)	
	Stage 4 n,%	3 (7.0)	4 (9.3)	
Cardiac status				
Mean LVEF % ±SD		52.5±13.4	51.4±12.4	0.684
LVEF category				0.667
	Good (LVEF > 55%) n,%	21 (48.5)	24 (55.8)	
	Moderate (LVEF 35–55%) n,%	18 (41.9)	14 (32.6)	
	Poor (LVEF < 35%) n,%	4 (9.3)	5 (11.6)	
No. diseased coronary arteries				0.331
	1 n,%	1 (2.3)	2 (4.7)	
	2 n,%	7 (16.3)	12 (27.9)	
	3 n,%	35 (81.4)	29 (67.4)	
Left main stem involvement n,%		7 (16.3)	9 (20.9)	0.579
Pre-operative drug history				
Aspirin n,%		41 (95.3)	42 (97.7)	1.000
Clopidogrel n,%		13 (30.2)	6 (14.0)	0.118
β blockers n,%		31 (72.1)	39 (90.7)	0.116
Ca^{2+} channel blockers n,%		16 (37.2)	16 (37.2)	1.000
Lipid lowering therapy n,%		40 (93.0)	39 (90.7)	1.000
ACE antagonist n,%		32 (74.4)	36 (83.7)	0.427
Long acting nitrates n,%		15 (35.0)	19 (44.2)	0.509
Potassium channel blockers n,%		2 (4.7)	2 (4.7)	1.000
Antidiabetic drugs	Insulin n,%	14 (32.6)	10 (23.3)	0.471
	Metformin n,%	12 (28.0)	17 (39.5)	0.362
	Sulphonylureas n,%	10 (23.3)	8 (18.6)	0.792

RIPC denotes remote ischaemic preconditioning; BMI body mass index; NYHA New York Heart Classification; MI myocardial infarction; TIA transient ischaemic attack; COPD chronic obstructive pulmonary disease; eGFR estimated glomerular filtration rate; sCr serum creatinine; CKD chronic kidney disease; LVEF left ventricular ejection fraction; ACE angiotensin converting enzyme.

Table 14 Operative characteristics of the study patients stratified by study group

Variable		RIPC (43 patients)	Control (43 patients)	p value
Surgical procedure				0.645
	CABG n,%	40 (93.0)	41 (95.3)	
	CABG+AVR n,%	3 (7.0)	2 (4.7)	
Procedural urgency				0.820
	Elective n,%	29 (67.4)	28 (65.1)	
	Urgent n,%	14 (32.6)	15 (34.9)	
Isoflurane anaesthesia n,%		37 (86.0)	38 (88.4)	0.747
Intermittent cross clamp fibrillation n,%		6 (14.0)	9 (20.9)	0.394
Number of grafts				0.762
	1 n,%	1 (2.3)	2 (4.7)	
	2 n,%	4 (9.3)	6 (14.0)	
	3 n,%	26 (60.5)	26 (60.5)	
	>3 n,%	12 (27.9)	9 (20.9)	
Cross-clamp time min±SD		66 (49 to 90)	58 (45 to 77)	0.092
Perfusion time min±SD		94 (78 to 119)	94 (74 to 123)	0.613
Input during operation				
	Crystalloid mL±SD	1993±1129	1826±376	0.875
	PRBC units±SD	0.302±0.118	0.279±0.096	0.879
Fluid input (0-24 hours) mL±SD		2566±946	3160±1583	0.202
Urine output (0-24 hours) mL±SD		2600±949	2920±1340	0.274
PRBC input (0-24 hours) mL±SD		0.97±0.29	1.32±0.25	0.152

RIPC denotes remote ischaemic preconditioning; CABG coronary artery bypass graft surgery; AVR aortic valve replacement; PRBC packed red blood cells.

4.3.1 Renal Outcomes

16/86 (18.6%) of patients developed post-operative AKI defined by RIFLE criteria. The incidence of AKI was not different between the RIPC and control group (RIPC 10/43 [23.3%] vs 6/43 [14.0%]; p=0.4065) (**Figure 9**). There was no significant difference in postoperative maximal increase in sCr between patients receiving RIPC and control (RIPC Δ SCr 110.3 [96.8 to 144.1] vs control Δ SCr 116.0 [108.3 to 127.4]; p=0.3509) (**Figure 9**).

In a post hoc analysis of patients who had AKI, 5 (11.6%) patients in each group had peak RIFLE category R; 3 (7.0%) and 1 (2.3%) patients had peak RIFLE category I; and 2 (4.6%) and 0 (0%) had peak RIFLE category F in the RIPC and control groups respectively (p=0.3577) (**Table 15**).

There was no difference in the incidence of AKI sustained for greater than 2 days (RIPC 5 [11.6%] vs control 2 [4.6%]) or 5 days (RIPC 2 [4.6%] vs control 1 [2.3%]) between the groups (p=0.7188) (**Table 15**). Finally, there was no difference in the need for renal replacement therapy between the groups; 2 (4.6%) patients in the RIPC group, compared with no (0%) patients in the control group (p=0.1524) (**Table 15**).

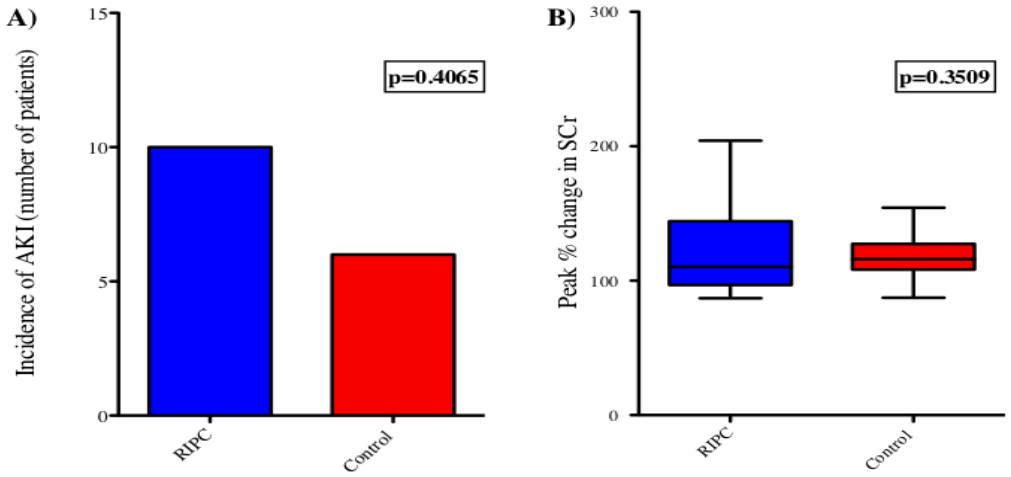


Figure 9 A) Incidence of AKI defined by a greater than 50% post-operative increase in sCr from pre-operative level. There was no statistically significant difference in the incidence of AKI between the RIPC and control groups (p=0.4065). **B) Absolute changes in plasma creatinine concentration after cardiac surgery from baseline to peak value at any time within the first five postoperative days.** Boxes represent 25th to 75th centiles, line median, bars 10th to 90th centiles.

Table 15 Post operative renal outcomes of study cohort stratified by study group

Variable	RIPC (43 patients)	Control (43 patients)	p value
Primary renal end-point			
Incidence of AKI n,%	10 (23.3)	6 (14.0)	0.268
Secondary renal end-points			
Δ SCr median (IQR)	110.3 (96.8 to 144.1)	116.0 (108.3 to 127.4)	0.351
Stage of AKI:			0.358
RIFLE max R n,%	5 (11.6)	5 (11.6)	
RIFLE max I n,%	3 (7.0)	1 (2.3)	
RIFLE max F n,%	2 (4.6)	0 (0)	
Duration of AKI:			0.605
1-2 days n,%	3 (7.0)	3 (7.0)	
3-4 days n,%	5 (11.6)	2 (4.6)	
>5 days n,%	2 (4.6)	1 (2.3)	
Incidence of post-operative RRT n,%	2 (4.6)	0 (0)	0.154

RIPC demotes remote ischaemic preconditioning; AKI acute kidney injury; sCr serum creatinine; RRT renal replacement therapy;

4.3.2 Serum Biomarkers

There was no difference in baseline, 6 h, 12 h or 24 h serum NGAL concentration (**Table 16**). Similarly, there were no differences in baseline, 6 h, 12 h or 24 h serum cystatin-C concentration or baseline, 6 h, 12 h, or 24 h serum IL-18 concentration (**Table 16**).

Table 16 Serum biomarker concentrations at 6 h, 12 h and 24 h after surgery stratified by study group

	RIPC (n=43)	Control (n=43)	P value
Serum NGAL concentrations (ng/ml)			
Baseline (IQR)	127.5 [74.5 to 179.5]	127.6 [79.5 to 178.0]	0.8830
6 hours post CABG (IQR)	256.2 [158.0 to 364.8]	207.7 [157.7 to 318.0]	0.5804
12 hours post CABG (IQR)	237.5 [159.6 to 407.9]	220.7 [174.5 to 314.0	0.5116
24 hours post CABG (IQR)	297.6 [166.8 to 440.4]	277.7 [191.7 to 351.2]	0.8022
Serum Cystatin-C (ng/ml)			
Baseline (IQR)	1376 [1163 to 1910]	1359 [1187 to 1746]	0.7493
6 hours post CABG (IQR)	1173 [938.5 to 1531]	1125 [913 to 1523]	0.7493
12 hours post CABG (IQR)	1330 [874 to 1769]	1326 [1070 to 1654]	0.7297
24 hours post CABG (IQR)	1486 [1103 to 2268]	1452 [1155 to 1936]	0.8089
Serum IL-18 (ng/ml)			
Baseline (IQR)	18.92 [12.94 to 23.21]	18.75 [13.89 to 23.82]	0.8022
6 hours post CABG (IQR)	14.26 [11.16 to 17.27]	15.13 [11.5 to 18.12]	0.7955
12 hours post CABG (IQR)	23.17 [18.65 to 27.32]	24.15 [18.26 to 29.26]	0.3737
24 hours post CABG (IQR)	30.76 [24.54 to 42.67]	32.2 [26.74 to 42.62]	0.5687

RIPC denotes remote ischaemic preconditioning; NGAL neutrophil gelatinase associated lipocalin; CABG coronary artery bypass graft surgery; IL-18 interleukin-18.

4.3.3 Urine Biomarkers

Urinary NGAL/urinary creatinine ratios were not different at baseline, 6 h, 12 h, or 24 h (**Table 17**). Similarly, no changes in baseline, 6 h, 12 h or 24 h urinary KIM-1/urinary creatinine ratio or baseline, 6 h, 12 h or 24 h urinary IL-18/urinary creatinine ratio were evident (**Table 17**).

Table 17 Urinary biomarker concentrations at 6 h, 12 h and 24 h after surgery stratified by study group

	RIPC (n=43)	Control (n=43)	P value
Urinary NGAL/urinary creatinine ratios (ng/mg)			
Baseline (IQR)	607.4 [269.5 to 2754]	633.6 [331.7 to 3100]	0.9518
6 hours post CABG (IQR)	4616 [2917 to 10764]	3550 [1431 to 12333]	0.7297
12 hours post CABG (IQR)	5673 [3250 to 11795]	5057 [1689 to 9851]	0.3691
24 hours post CABG (IQR)	2783 [812.5 to 11191]	2760 [867.3 to 8647]	0.8969
Urinary KIM-1/urinary creatinine (pg/mg)			
Baseline (IQR)	988.1 [471.1 to 1530]	908.7 [471 to 1463]	0.9656
6 hours post CABG (IQR)	586.9 [305.3 to 1119]	744.5 [338.3 to 1284]	0.2615
12 hours post CABG (IQR)	1389 [767.8 to 2531]	1585 [732.1 to 2956]	0.7889
24 hours post CABG (IQR)	1470 [931.8 to 2488]	1207 [695.1 to 3032]	0.6409
Urinary IL-18/ urinary creatinine ratio (ng/mg)			
Baseline (IQR)	1280 [522.2 to 2244]	881.7 [330.1 to 2291]	0.4576
6 hours post CABG (IQR)	403.6 [148.8 to 897.3]	344.9 [96.26 to 969.5]	0.9589
12 hours post CABG (IQR)	716.9 [197.8 to 3003]	534.1 [126.8 to 1750]	0.2803

RIPC denotes remote ischaemic preconditioning; NGAL neutrophil gelatinase associated lipocalin; CABG coronary artery bypass graft surgery; KIM-1 kidney injury molecule-1; IL-18 interleukin-18.

4.3.4 Cardiac Outcomes

Of 516 possible cTnT measurements, all were assayed and available for subsequent analysis. There was no difference between the groups in terms of the primary cardiac end-point of 72 h AUC cTnT release (RIPC, 34686 [23838 to 57768] vs control 31269 [22374 to 41958] ng/L/72 h; p=0.3668) (**Figure 10**). Furthermore, 6 h cTnT (RIPC 851 [532 to 1316] vs control 758 [560 to 1036] ng/L; p=0.4046) and peak cTnT (RIPC 851 [558 to 1407] vs control 842 [607 to 1344] ng/L; p=0.7168) were not different between the groups. Finally, the incidence of post-operative myocardial infarction (RIPC 3/43 vs control 2/43; p=1.0) and post-operative atrial fibrillation (RIPC 13/43 vs 15/43; p=0.8183) (**Table 18**) were not different between the groups.

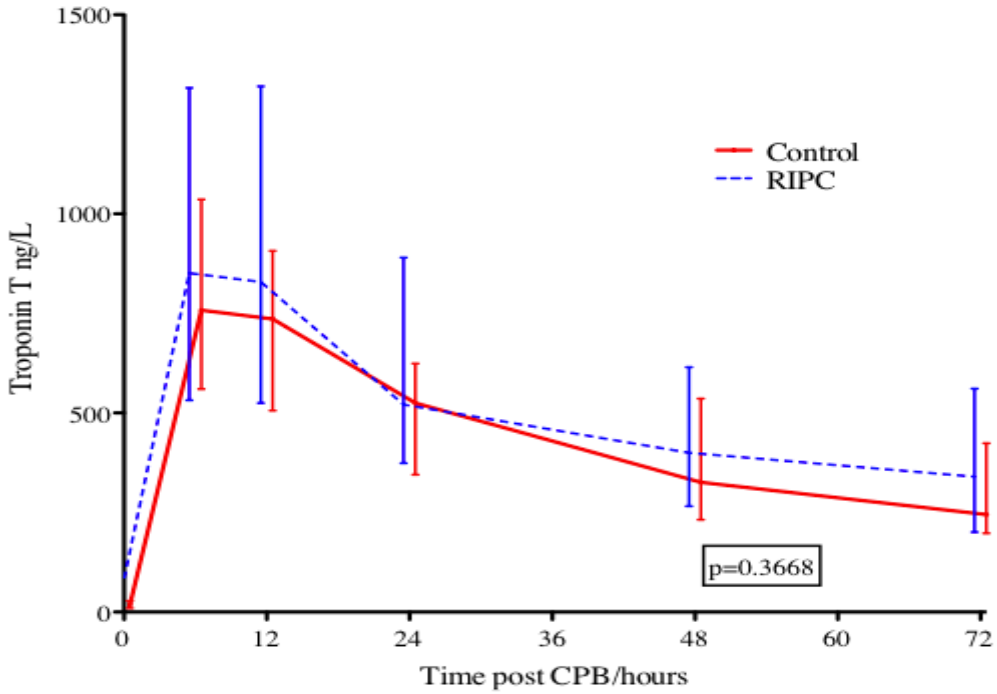


Figure 10 Cardiac troponin T release over 72 hours. Medians and interquartile ranges are presented. No difference in release profiles were identified (p=0.3668).

4.3.5 Other postoperative outcomes

There was no significant difference between groups in the use inotropes over the first 24 h, or the duration of mechanical ventilation. Length of intensive care unit stay and hospital stay after surgery were comparable between the groups. Two patient in the control group and 2 patients in the RIPC group died within 30 days of surgery (**Table 18**).

Table 18 Post operative outcomes of study cohort stratified by study group

Variable	RIPC (43 patients)	Control (43 patients)	p value
Primary cardiac end-point			
72 hours cTnT AUC ng/L (IQR)	34686 (23838 to 57768)	31269 (22374 to 41958)	0.367
Secondary cardiac end-points			
6-hour cTnT (IQR)	851 (532 to 1316)	758 (560 to 1036)	0.405
Peak cTnT (IQR)	851 (558 to 1407)	842 (607 to 1344)	0.717
Incidence of MI n, %	3 (7.0)	2 (4.6)	1.000
Incidence of AF n, %	13 (30.2)	15 (34.9)	0.818
Other outcomes			
Inotrope use n, %	31 (72.1)	34 (79.1)	0.451
Inotrope score 0-24 hours (IQR)	64.5 (0 to 201)	63 (3 to 180)	0.882
Extubation time, min (IQR)	483 (530 to 885)	720 (395 to 930)	0.239
ICU length of stay, h (IQR)	22.4 (14.4 to 31.8)	22.0 (17.3 to 47.8)	0.242
Hospital length of stay days, %	8 (6 to 12)	8 (6 to 12)	0.512
30-day mortality n, %	2 (4.6)	2 (4.6)	1.000

RIPC demotes remote ischaemic preconditioning; cTnT AUC cardiac troponin T area under the curve; MI myocardial infarction; AF atrial fibrillation; ICU intensive care unit.

4.3.6 Sub-group analysis

When the analysis of primary end-points was repeated in the 31 non-diabetic patients (16 RIPC and 15 control) who underwent surgery there was no difference in 72 h AUC cTnT release (RIPC 30153 [19045 to 52501] vs control 33999 [23397 to 38355] ng/L/72 h; p=0.4526), or the incidence of AKI (RIPC 2/16 [12.5%] vs control 2/15 [13.3]; p=1.000) between the groups. Similarly, in elective surgical patients (29 RIPC and 28 controls) 72 h AUC cTnT release (RIPC 36699 [27072 to 58412] vs control 30981 [22630 to 41447] ng/L/72h; p=0.1698) and incidence of AKI (RIPC 3/29 [10.3%] vs 5/28 [17.9%]; p=0.4703) was not different between the groups. Finally, in patients undergoing surgery with cardioplegia (37 RIPC vs 34 controls) RIPC had no effect upon 72 h AUC cTnT release (RIPC 34677 [23391 to 58412] vs control 30645 [21293 to 40426] ng/L/72h; p=0.2844) or the incidence of AKI (RIPC 9/37 [24.3%] vs 5/34 [14.7%]; p=0.3789) when compared to controls.

Importantly, it must be stated that analyzing sub-groups in such a small study is fraught with difficulties. Even though these sub-groups were pre-specified they are small and underpowered to provide any definitive statistical results.

4.4 Discussion

The present randomized controlled study investigates whether RIPC induced by intermittent forearm ischaemia in the anaesthetic room immediately before cardiac surgery attenuates subsequent myocardial or renal injury sustained during CPB in patients with established CKD. There was no evidence to suggest that RIPC reduces the incidence of AKI, assessed by either RIFLE criteria or changes in biomarker of renal injury. Furthermore RIPC had no effect upon myocardial enzyme release or other important clinical end-points including length of ITU or hospital admission and 30-day mortality.

AKI is a significant problem following cardiac surgery. Consistent factors implicated in the development of AKI following cardiac surgery include advanced age, diabetes mellitus, congestive heart failure, need for emergency surgery, surgical complexity and most importantly pre-operative CKD [54, 98, 100, 107]. Once AKI has occurred, it is associated with an increase in subsequent mortality [6, 99, 100, 106, 113, 171]. Mortality is highest in patients with AKI that requiring post-operative dialysis, and may exceed 60% [100], however, smaller transient post-operative increases in sCr without obvious immediate clinical sequelae are also associated with an increase in both early and late mortality [6, 113]. As shown in chapter 3 the relationship between AKI and excess mortality persist even after adjusting for patient comorbidity and surgical complexity [6, 113].

Despite advances in our understanding of the aetiology and the pathophysiological processes central to the development of AKI following cardiac surgery, neither the incidence nor the mortality associated with this condition have changed [108]. Currently no established prophylaxes or therapies for AKI exist [133] and novel methods to prevent and manage this complication are now urgently needed to improve outcomes. RIPC describes a phenomenon by which the application of brief non-lethal ischaemia and reperfusion injury to an organ protects a distant organ from a subsequent episode of prolonged ischaemia and reperfusion. RIPC induced using transient limb ischaemia has been reported to reduce perioperative myocardial enzyme release in both adults and children undergoing cardiac surgery [198, 199]. RIPC has also been reported to afford renal protection in patients undergoing vascular surgery

[144], coronary angiography [216], and cardiac surgery [148]. Due to their particularly high incidence of post-cardiac surgical AKI, patients with CKD may accrue significant benefit from RIPC. Whether RIPC is efficacious in patients with CKD is unknown as it not previously been studied selectively in such a cohort. For a variety of reasons patients with CKD may be 'resistant' to the organ protection generated using RIPC. Our research group found that uraemia does not attenuate the affect of RIPC in a rodent model of myocardial infarction [214]. This finding stimulated our hypothesis that RIPC may offer important renal protection and myocardial protection in patients with CKD undergoing cardiac surgery.

Most previous studies of RIPC have been undertaken in low-risk patients undergoing isolated CABG surgery. These patients are least likely to accrue any meaningful benefit from RIPC. For RIPC to be translated into a useful clinical adjunct in cardiac surgery it must be proven within a 'high-risk' surgical cohort such as patients with CKD.

RIPC had no effect upon the incidence of AKI defined by the RIFLE criteria. AKI defined using these criteria has been associated with both short and long term complications after cardiac surgery [6]. These criteria are largely based upon peri-operative changes in sCr. sCr has several limitations as a marker of AKI; firstly sCr can be affected by age, gender, diet, muscle mass, and some drugs. Secondly, important changes in glomerular filtration may be masked as up to 40% of creatinine clearance is due to the renal secretion of creatinine. Finally, sCr levels only become abnormal when greater than 50% of glomerular filtering capacity is lost, and it may require up to 24 h following AKI for increases in sCr to become evident. For this reason we also assessed AKI using novel biomarkers of renal injury; namely serum and urine NGAL, serum and urine IL-18, serum Cy-C and urinary KIM. These biomarkers have been reported to be sensitive, specific surrogates of renal injury and potentially may be useful to detect small difference in renal outcome in interventional studies [217]. We found no effect of RIPC upon the post-operative levels of these biomarkers of renal injury during the first 24 h after cardiac surgery.

Perioperative myocardial injury during cardiac surgery was assessed by serial cTnT measurements over the first 72 h following surgery. This is a standard end-point in cardiac RIPC studies. Early post-operative cTn elevation is associated with an increased early and late mortality following cardiac surgery[218]. Conceptually, improved myocardial protection reduces cTn release, and may translate into improved post surgical clinical outcomes. We

found no effect of RIPC upon cardiac troponin release following cardiac surgery in patients with CKD.

A potential criticism of the study methodology is that cTn release and excretion is altered in end stage renal disease (ESRF). CTn elevation in the absence of myocardial injury is common in patients with ESRD and identifies patients at risk of premature cardiac death [219]. However, cTn is not cleared by the kidney, and lesser degrees of renal dysfunction are not associated with cTn elevation in the absence of cardiac disease [220]. ESRD represented a study exclusion, and the majority (>90%) of patients in the study cohort had lesser degrees of renal dysfunction (eGFR 30-60 mL/min). Thus it seems cTn is a legitimate surrogate marker of myocardial injury in the study cohort. It should be noted that 2 patients in the RIPC group required renal replacement therapy. It is possible that cTnT AUC in these patients was altered as certain haemodialysis filters can affect the clearance of cTnT. Alternative surrogates of perioperative myocardial injury that may have been used include serum CK-MB but this is subject to many of the same issues as cTn in patients with CKD[221], and/or LGE-CMR. Delayed LGE washout of infarcted myocardium allows CMR to detect area of confluent infarcted myocardium as small as 1 gram [222]. We had initially planned to use LGE-CMR as a secondary cardiac end-point but the use of LGE-CMR was prevented by concerns of administering gadolinium to patients with an eGFR < 30 mls/min or recent AKI.

Following cardiac surgery myocardial IRI may be manifest as cardiac stunning; a transient period of reversible myocardial contractile dysfunction. Clinically cardiac stunning is treated by optimizing haemodymanics with temporary inotropic support. There was no difference in inotrope requirements over the first 24 hours post-operatively suggesting that RIPC did not effect cardiac stunning in this cohort. Finally RIPC did not effect time on intensive care, hospital length of stay or 30-day mortality, although obviously the study was underpowered to detect any differences in these clinical parameters.

There are a number of potential explanations for the lack of organ protection afforded by RIPC in this study. Firstly, comorbidity including hypertension, hyperlipidaemia, and heart failure may render organs resistant to RIPC [209, 211]. As these comorbidities are frequently present in patients with CKD this cohort may be particularly resistant to RIPC. Furthermore, diabetes (also especially prevalent in our study cohort) and antidiabetic medication may interfere with the signal transduction pathways believed to mediate RIPC thus reducing efficacy of RIPC [210]. Secondly, by recruiting patients undergoing cardiac surgery post MI,

we may have reduced any measurable effect of RIPC. Although MI within 1 week of surgery represented a study exclusion, it is conceivable that a recent MI may have conferred some degree of subsequent organ protection by a mechanism similar to RIPC, making any beneficial organ protection afforded by RIPC more difficult to detect. Finally, anaesthetic and surgical technique may affect RIPC organ protection. Volatile anaesthetics, propofol and intravenous nitrates can independently mediate organ protection [207, 208]. Most negative RIPC studies have allowed the use of volatile anaesthetics, and this is often quoted as a potential reason for the failure to demonstrate a beneficial effect of RIPC [141]. Also, intermittent cross-clamp fibrillation may offer direct preconditioning of the myocardium. A single surgeon working at Barts Health NHS Trust still uses this technique. Potentially the use of intermittent cross-clamp fibrillation could reduce any additional organ protection conferred by RIPC thus confounding our results. We chose not to standardise anaesthetic or surgical regimens in order to maintain the clinical relevance of this study. For RIPC to be adopted into modern cardiac surgical practice it must offer benefit above and beyond currently used renal and cardiac protective therapies.

4.4.1 Limitations

This was a single blinded study. Patients, anesthetists, surgeons and critical care staff were blinded to study group allocation. However for logistical reasons researchers were not blinded. As RIPC represents a binary intervention and our cardiac and renal outcomes are clearly objective, it is unlikely that researchers could introduce any significant bias into the study.

The RIPC stimulus used in this study was 3 cycles of 5 min forearm ischaemia induced in the anaesthetic room immediately before surgery. This is a standard preconditioning protocol used in many previous studies of RIPC. It is possible that patients with CKD have a higher 'conditioning threshold' and thus the stimulus used here was submaximal. The only prospective cardiac surgical study to demonstrate renal protection with RIPC used leg ischaemia as the preconditioning stimulus [144, 148]. Using leg ischaemia or, more or longer cycles of forearm ischaemia and reperfusion may have been more appropriate in a potentially 'RIPC resistant' cohort.

This was a pragmatic study. No 'meaningful' clinical effect of RIPC upon the renal or myocardial end-points studied was evident. However, this is different to concluding that RIPC has no effect in this setting. The *a priori* assumption was that RIPC would reduce

myocardial enzyme release by 25%. Failure to achieve this degree of cardioprotection means that our study is underpowered to provide definitive statistical results. Furthermore, the lack of standardized anaesthetic and surgical protocols may have prevented any effect of RIPC from becoming evident. But as stressed through out, for anaesthetists and surgeons to adopt RIPC into daily practice it must be a useful adjunct to current standard therapies, rather than an awkward and time consuming alternative.

4.5 Conclusions

RIPC conferred no evident additional myocardial or renal protection beyond current standard anaesthetic and surgical management of patients with CKD undergoing cardiac surgery.

Chapter 5

5. Comparative analysis of biomarkers for the early detection of cardiac surgery associated – acute kidney injury in patients with CKD

Introduction

Acute kidney injury (AKI) is a common and serious complication of cardiac surgery. We have investigated the accuracy of 3 serum (NGAL, IL-18 and CyC) and 3 urinary (NGAL, IL-18 and KIM-1) biomarkers for predicting AKI in patients with CKD undergoing cardiac surgery.

Methods

AKI biomarkers were measured at 6, 12 and 24 hours after cardiac surgery in 86 patients with CKD undergoing cardiac surgery. AKI was defined as a ≥50% increase in serum creatinine from preoperative levels. AUC-ROC curves were constructed to assess sensitivities and specificities for each biomarker.

Results

Sixteen patients (18.6%) patients developed AKI. The best predictor of AKI at all time points was percentage change in serum CyC level displaying the highest AUC-ROC at 24 hours after CABG (AUC-ROC 0.94 [0.89-0.99]). Percentage change in serum NGAL was also a fair predictor of AKI at all time points with best performance at 24 hours (AUC-ROC 0.83 [0.74-0.93]). Serum IL-18, and all of the urinary biomarkers studied were poor predictors of AKI.

Conclusions

Change in serum CyC and serum NGAL over the first 24 hours after cardiac surgery were useful predictors of CSA-AKI in adults with CKD after cardiac surgery. Urinary biomarkers of CSA-AKI were less useful as early predictors of CSA-AKI in this cohort.

5.1 Introduction

As I have detailed throughout this thesis, despite a myriad of research in the area no prophylactic intervention has been definitively shown to reduce the incidence of AKI following cardiac surgery. Despite the theoretical promise of RIPC, this intervention also failed to modify AKI in our surgical cohort.

Rather than relying solely upon prophylaxis, therapeutic modification of confirmed AKI may be possible. Preclinical studies have demonstrated that pharmacological treatment of AKI is effective, but only if therapies are initiated early after the renal insult [223-226]. The current clinical diagnosis of AKI is based upon changes in sCr. SCr is an indicator of renal function, and not a marker of renal injury. Concentrations of sCr do not change until approximately 50% of renal function is lost, and an increase in sCr diagnostic of AKI may lag by as much as 3 days behind the initial renal insult. Attempts to modify AKI at this late stage are unlikely to be effective as renal tubular injury is already established. This lag time between the renal injury and the resultant loss of function finally reflected as an elevation of sCr is a missed therapeutic opportunity. An alternative to sCr that allows the early diagnosis of AKI is now urgently needed if novel therapies to treat AKI are to be developed.

The American Society of Nephrology has designated identification and development of new AKI biomarkers as a key research objective [227]. The ideal AKI biomarker would allow early identification of renal injury, stratify the severity of renal injury, characterization of the location and/or aetiology of injury, provide prognostic information and allow the monitoring of response to therapy. However, due to heterogeneity of AKI it unlikely that any single AKI biomarker can achieve all of these properties. Thus it seems likely that more than one AKI biomarker will be needed and combination of AKI biomarkers in a ‘kidney injury’ panel will be necessary.

With the application of functional genomics and proteomics to human and animal AKI models several potential AKI biomarkers have been identified. The most promising of these include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18). These biomarkers have the potential to revolutionise care for patients with AKI. Below I will review the evidence supporting the use of these AKI biomarkers for the prediction of AKI in cardiac surgery.

5.1.1 Neutrophil gelatinase-associated lipocalin:

In health NGAL is barely detectable in serum or urine. It mediates iron transport to the proximal tubular cells, where it exerts a protective bacteriostatic effect, binding to and inactivating iron gathering molecules (siderophores) synthesised by bacteria, and an antioxidant effect, gathering free iron to prevent production of ROS that may result in oxidative stress and renal tubular injury [228]. In response to acute tubular injury NGAL is excreted in huge quantities, becoming readily detectable in both serum and urine more than 24 hours prior to any rise in sCr [228]. It is this characteristic that enables NGAL to serve as a potential biomarker of AKI.

The first clinical study evaluating NGAL as an AKI predictor was in children at risk of AKI after cardiac surgery. At between 2 and 6 h post CPB both urine and plasma NGAL were excellent predictors of the subsequent development of CSA-AKI, demonstrating area under the receiver-operating characteristic curve (AUC- ROC) of >0.9 [229]. NGAL has proven a less impressive predictor AKI in adult cardiac surgical cohorts [230]. Although NGAL is demonstrated in significant quantities in both urine and plasma within 3 h of surgery in patients subsequently diagnosed with AKI, the AUC-ROC for AKI prediction has varied widely from 0.61 to 0.96 in published studies [203, 217, 231-233]. The limited ability of NGAL to predict AKI in adult cardiac surgery may in part be due to the increased burden of comorbidity seen in these patients. For example diabetes [234] and CKD [235] may affect the diagnostic performance of NGAL. Moreover, the non-uniform definitions of AKI, different NGAL sampling times and detection techniques used in some of these studies are also likely to have affected the performance of NGAL as a predictor of AKI [236]. Despite this a recent meta-analysis of published adult cardiac surgical studies (10 studies 1204 patients) reported an overall AUC-ROC of 0.76 (95% CI 0.70 to 0.82) for prediction of AKI (defined as a >50% increase in sCr) when either urinary or plasma NGAL was measured within 6 h of initiation of CPB [231]. This

performance compares favorably with that of cTn for the prediction of MI during its clinical implementation period [231].

5.1.2 Cystatin C:

CyC is a low molecular weight cysteine protease inhibitor that is produced by all nucleated cells. In health CyC is freely filtered at the glomerulus. It is neither secreted nor reabsorbed by renal tubules but is taken up by proximal tubule cells where it is completely catabolized. Consequently the CyC concentration in urine and serum is negligible. As CyC levels are not significantly affected by age, gender, race, or muscle mass, and it is not secreted by the renal tubules, CyC is probably a better measure of glomerular function than sCr [237]. After renal tubular injury GFR falls leading to an increase in serum CyC levels. Also urinary CyC concentration increases due to decreased reabsorption of CyC in the proximal tubule [237].

Serum CyC appears to be an extremely promising AKI biomarker. It reaches peak serum concentration approximately 10 h later than NGAL at around 12-24 h post CPB [227]. A recent meta-analysis (9 cardiothoracic surgical studies comprising 1330 adult and paediatric patients) reported that CyC was a good predictor of AKI with an AUC-ROC 0f 0.78 – 0.96 if measured within 12 h of CPB [238].

5.1.3 Interleukin 18:

IL-18 is a proinflammatory cytokine and powerful modulator of ischaemic AKI. In response to renal injury IL-18 is induced and cleaved in the proximal tubule becoming detectable in urine [237]. In human studies IL-18 appears a specific marker of AKI not detectable in the urine of patients with CKD, or urinary tract infections [237].

To date urinary IL-18 has proved at best a moderate predictor of CSA-AKI. In 3 studies enrolling 258 adults, the AUC-ROC for urinary IL-18 at admission to intensive care for the prediction of CSA-AKI ranged from 0.53 to 0.66 [239-241]. Importantly IL-18 levels peak approximately 6 - 12 h post CPB, and so early sampling time may potentially explain the disappointing results of these studies. When combined with other AKI biomarkers IL-18 has yielded more promising results [240].

5.1.4 Kidney injury molecule-1:

KIM-1 is a transmembrane glycoprotein, not usually expressed in healthy kidneys or urine. KIM-1 is important modulator of the immune response and repair processes of the injured kidney. Following ischaemic or toxic renal injury, KIM-1 expression is up regulated, transforming surviving proximal renal epithelial cells into phagocytes that may assist with the clearance of apoptotic and necrotic cells resulting from AKI [237]. After renal injury the ectodomain segment of KIM-1 becomes detectable in urine (with levels peaking 12 to 24 h after the renal insult), thus stimulating interest in urinary KIM-1 as a potential biomarker of proximal renal tubular injury [227].

Although in non-cardiac surgical cohorts several groups have found that KIM-1 is a sensitive indicator of AKI, KIM-1 has been less intensively investigated than NGAL or CyC in cardiac surgery. A meta-analysis (8 studies between 2002 to 2009) evaluating the performance of urinary KIM-1 in cardiac surgery found that within 24 hours of CPB the sensitivity of KIM-1 for detecting CSA-AKI ranged from 92% to 100% and the AUC-ROC from 0.78 to 0.91 [242].

In summary, there are currently a number of promising candidate AKI biomarkers to facilitate the early diagnosis of AKI after cardiac surgery. To date the diagnostic performance of these biomarkers in a population of cardiac surgical patients with CKD is untested. As these patients represent those at the greatest risk of developing AKI, and they are accounting form an increasing proportion of patients undergoing cardiac surgery each year, the development of biomarkers that can reliably differentiate those patients that will develop AKI at an early post-operative time point would constitute a significant advance. Potentially novel treatments for AKI could then be instituted within a time frame when AKI can be modified.

This chapter describes the secondary analysis of the randomized control trial to assessing RIPC in patients with CKD undergoing CABG. The aim of this analysis was to evaluate the diagnostic performance of a number of serum and urine AKI biomarkers within cohort of cardiac surgical patients with established CKD.

5.2 Methods

This is a secondary analysis of the trial described in chapter 4. In brief, this was a randomised, controlled, single centre study of the effect of RIPC upon myocardial and renal injury in 86 patients with non-dialysis dependent CKD undergoing CABG with or without AVR. No effect of RIPC upon either AKI or myocardial injury was evident.

5.2.1 Study Protocol

Study enrollment is described in chapter 4. 86 patients with CKD (eGFR < 60 mL/min) were enrolled. Detailed demographic, clinical and surgical data were recorded for all patients as previously described.

Blood samples for sCr were drawn pre-operatively and then daily for 5 days post-operatively unless patients were discharged within this period. Blood and urine samples for biomarkers of renal injury were obtained pre-operatively and then post–operatively at 6, 12 and 24 h after separation from CPB. Both blood and urine samples for biomarkers were centrifuged at 1300 rpm for 15 minutes. Serum and urinary supernatant were removed and stored at -80ºC until biomarker assay.

AKI was defined in accordance with RIFLE criteria as any post-operative increase in sCr > 50% from the preoperative value within 5 days of cardiac surgery [110].

5.2.2 Biomarker analysis

Serum NGAL, IL-18 and cystatin C, and urinary NGAL, IL-18 and KIM-1 were assessed. All biomarkers were measured using commercially available enzyme-linked immunoabsorbant assay (ELISA) kits in accordance with the manufacturers instructions.

NGAL (ELISA): A commercially available ELISA kit (Human Lipocalin-2/NGAL Immunoassay; R&D Systems, Europe) was used to measure NGAL concentration in serum and urine samples. The protocol for measurement of NGAL concentration is detailed in appendix 1. 2.

IL-18 (ELISA): IL-18 concentration in serum and urine were measured using a commercially available ELISA kit (Human IL-18 BPa Immunoassay; R&D Systems, Europe). The detailed protocol for measurement of IL-18 concentration is available in appendix 1.3.

CyC ELISA: CyC concentration in serum was measured using a commercial ELISA kit (Human Cystatin C Immunoassay; R&D Systems, Europe). The protocol for cystatin C measurement is detailed in appendix 1.4.

KIM-1 ELISA: A commercially available ELISA kit (TIM-1/KIM-1/HAVCR Immunoassay; R&D Systems, Europe) was used to measure KIM-1 concentration in urine samples. The protocol for measurement of KIM-1 concentration is detailed in appendix 1.5.

All measurements were performed in duplicate. All urinary biomarker results were normalized to urinary creatinine values, by dividing urinary biomarker concentration (ng/mL) by the urinary creatinine concentration (mg/mL) and expressed as ng/mg of creatinine.

Urinary creatinine assay: Urinary creatinine was detected by using a commercially available fluorometric assay (Creatinine Assay; Abcam). The protocol for urinary creatinine measurement is detailed in appendix 1. 5.

5.2.3 Statistical Analysis

Patients were divided into those that sustained AKI (AKI group) and those that did not (no AKI group) for further analysis (**Figure 11**). Normality of distribution of continuous data was assessed using the Shapiro-Wilks test. Normally distributed continuous data are presented as mean (±SD); whereas skewed continuous data are presented as median (interquartile range [IQR]). Normally distributed data were compared with a Students t-test and non-normally distributed data were analysed non-parametrically (Mann-Whitney U test). Categorical data are summarized using absolute values (percentage). Categorical data were compared using the Pearson chi-square test or Fishers exact test where appropriate.

Individual biomarker performance characteristics for the detection of AKI were evaluated with receiver operator characteristic (ROC) curve analysis. There was substantial variability between individual patients in absolute biomarker concentrations at baseline and subsequent time points. Therefore, we chose to assess the performance of biomarkers using percentage change in concentration rather than absolute value. Optimal cut-off points for predicting AKI for each biomarker were determined using the Youden index, which is calculated by adding sensitivity and specificity and subtracting 1 [243]. This identifies the point on the ROC curve that has the maximum vertical distance to the chance line. Biomarkers were dichotomized using these cut-offs to allow sensitivity, specificity, negative predictive value and positive predictive value for each biomarker to be calculated. In addition, serum and urine biomarkers with the best area-under-the-ROC curve (AUC-ROC) at 6 and 12 h were combined in order to assess the value of combination biomarker panels for the prediction of AKI.

Differences were considered statistically significant at a $p < 0.05$. Statistical analyses and figure preparation were undertaken using SPSS (Version 18.0, IBM SPSS statistics UK) and GraphPad Prism 5 for MacOS (GraphPad Software, San Diego, CA, USA).

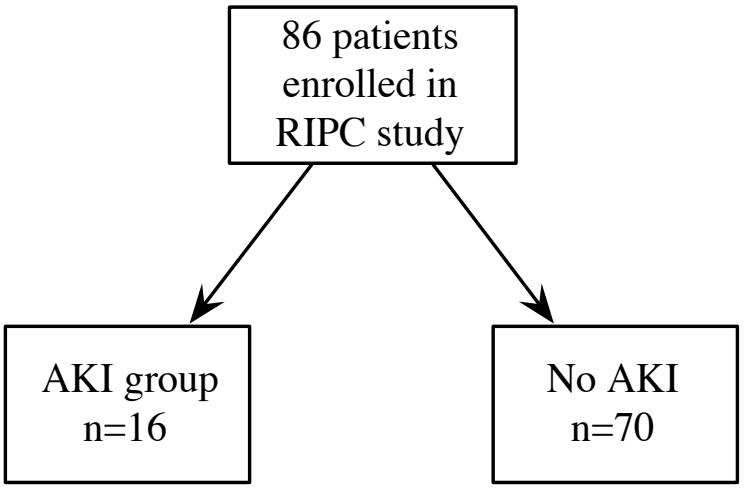


Figure 11 Study flow chart. Patients were divided into those that sustained acute kidney injury (AKI group; n=16) and those that did not sustain acute kidney injury (no AKI; n=70) for analysis.

5.3 Results

5.3.1 Characteristics of the study cohort

The clinical characteristics of the study cohort stratified by development of AKI are displayed in **Table19**. The surgical characteristics of the study cohort are displayed in **Table 20**.

The incidence of AKI in the study cohort was 18.6% (16/86). There was no significant difference in any clinical or surgical characteristic between those that developed AKI and those that did not. Post-operatively there was a trend towards increased length of ITU (32.2 vs 21.3 h; p=0.058) and total hospital stay (13.0 vs 7.5 day; p=0.053) in patients that developed AKI. Also, post-operative dialysis (2/16 vs 0/70; p=0.033) and 30-day mortality (2/16 vs 0/70; p=0.033) were significantly higher in those patients that developed AKI.

Table 19 Baseline demographics and clinical characteristics of the study cohort stratified by the presence or absence of AKI

Variable	AKI (16 patients)	No AKI (70 patients)	p value
Demographics			
Age(years)	75.5 (57.3 to 81.3)	71.0 (64 to 77.25)	0.534
BMI (kg/m^2)	25.7 (23.2 to 29.4)	27.9 (24.7 to 30.6)	0.267
Gender (female)	2 (12.5)	15 (21.5)	0.418
Ethnicity			0.412
Caucasian	9 (56.3)	48 (56.3)	
Afro-Caribbean	2 (12.5)	3 (4.3)	
South Asian	5 (31.3)	16 (22.9)	
Pre-operative Symptom status			
NYHA class > 3	6 (37.5)	23 (32.9)	0.851
Clinical characteristics			
Diabetes Mellitus (DM)			0.469
Non-insulin treated DM	6 (37.5)	26 (37.1)	
Insulin treated DM	6 (37.5)	17 (24.3)	
Hypertension	15 (93.8)	56 (80.0)	0.191
Hypercholesterolaemia	15 (93.8)	52 (74.3)	0.090
Previous MI	11 (68.8)	34 (48.6)	0.145
Previous stroke	4 (25.0)	11 (15.7)	0.377
Peripheral vascular disease	4 (25.0)	25 (35.7)	0.413
COPD	1 (6.3)	8 (11.4)	0.542
Pre-operative Renal Function			
Median eGFR (mL/min)	47.5 (38.5 to 52.8)	51.0 (43.8 to 56.0)	0.161
Median sCr (μmol/L)	124.0 (114.0 to 155.3)	121.0 (110.5 to 137.8)	0.421
Cardiac status			
Mean LVEF (%)	47.5 (39.3 to 57.5)	56.0 (45.0 to 60.0)	0.113
LVEF category			0.215
Good (LVEF > 55%)	6 (37.5)	39 (55.7)	
Moderate (LVEF 35 – 55%)	9 (56.3)	23 (32.9)	
Poor (LVEF < 35%)	1 (6.3)	8 (11.4)	
3 vessel coronary artery disease	15 (93.8)	47 (70.0)	0.142
Left main stem involvement	1 (6.3)	15 (21.4)	0.159

BMI denotes body mass index; NYHA New York Heart Association; DM diabetes mellitus; MI myocardial infarction; eGFR estimated glomerular filtration rate; sCr serum creatinine; LVEF left ventricular ejection fraction.

Table 20 Surgical characteristics and post-operative outcomes of the study cohort stratified by the presence or absence of AKI

Variable	AKI (16 patients)	No AKI (70 patients)	p value
Surgical procedure			0.271
CABG	16 (100.0)	65 (92.9)	
CABG + AVR	0 (0)	5 (7.1)	
Procedural urgency			0.127
Elective	8 (50.0)	49 (70.0)	
Urgent	8 (50.0)	21 (30.0)	
Number of grafts			0.161
1	0 (0)	3 (4.3)	
2	0 (0)	10 (14.3)	
3	14 (87.5)	38 (54.3)	
>3	2 (12.5)	19 (27.1)	
Cross-clamp time (min)	97.5 (75.8 to 119.0)	93 (76.0 to 119.3)	0.863
Perfusion time (min)	63.5 (57.3 to 83.8)	60.0 (46.8 to 89.0)	0.363
Post-operative outcomes			
Post-operative dialysis	2 (2.3)	0 (0)	0.033
Myocardial infarction	0 (0)	5 (7.1)	0.271
Atrial fibrillation	7 (43.8)	21 (30.0)	0.290
Pneumonia	2 (12.5)	1 (1.4)	0.088
Need to return to theatre	1 (6.3)	2 (2.9)	0.505
Length of ICU stay (h)	32.2 (21.3 to 56.7)	21.3 (16.8 to 32.4)	0.058
Length of hospital stay (day)	13.0 (6.5 to 17.5)	7.5 (6.0 to 11.0)	0.053
30-day mortality	3 (18.8)	0 (0)	0.006

CABG denotes coronary artery bypass graft surgery; AVR aortic valve replacement; ICU intensive care unit

5.3.2 Serum creatinine after CABG (Figure 12)

There was no difference in median pre-operative sCr levels between the AKI and no AKI groups (124 μmol/L vs 121 μmol/L; p=0.421). By post-operative day 1 a significant separation in sCr levels between the AKI and no AKI groups became evident, and this was sustained until post-operative day 5. AKI defined by RIFLE criteria was evident after a median of 2 days (2 to 3) in those that sustained AKI.

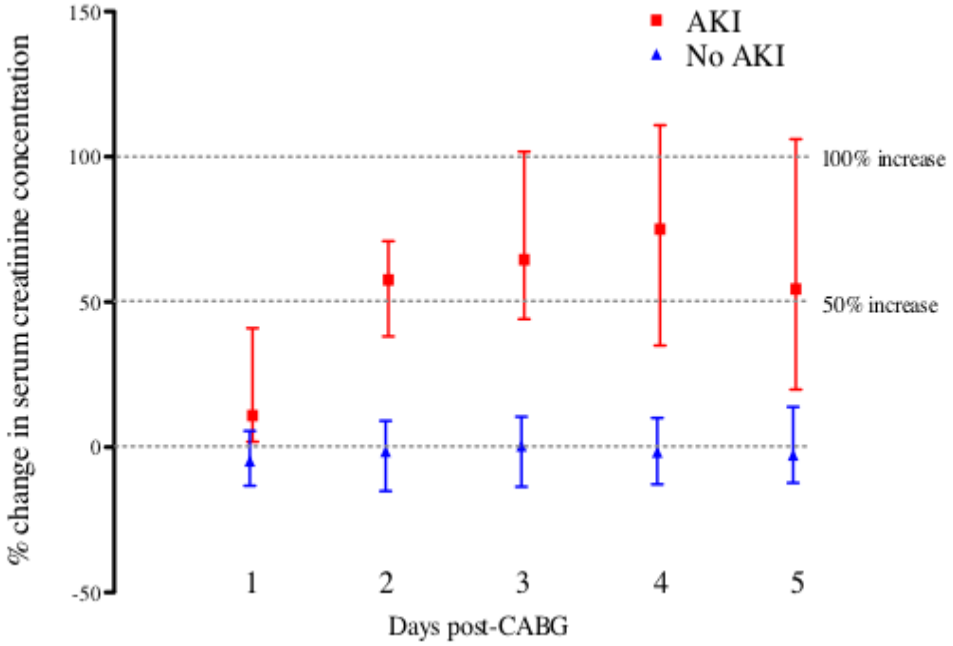


Figure 12 Median % increase in serum creatinine over the first 5 post-operative days stratified by the presence or absence of AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. There was no difference in baseline sCr between patients with no AKI and patients with AKI (121.0 vs 124.0; p=0.4209). On each post operative day the AKI group have a higher % increase in serum creatine than the no AKI group; day 1 (no AKI -4.7% vs AKI 10.9%; p=0.0007), day 2 (no AKI -1.4% vs AKI 57.6%; p<0.0001), day 3 (no AKI 0.5% vs AKI 64.6%; p<0.0001), day 4 (no AKI -1.7% vs 75.1%; p<0.0001) and day 5 (no AKI -2.7% vs AKI 54.5%; p<0.0001).

5.3.3 Serum AKI biomarkers after CABG (Figure 13)

NGAL: Pre-operative serum NGAL concentration was not different between the groups (AKI 130.9 [87.9 to 214.5] vs 108.5 [72.9 vs 172.1] ng/mL; p=0.2507). Percentage change in serum NGAL was significantly higher in the AKI group at 6 h (AKI 116.1% vs No AKI 69.5%; p=0.0046), 12 h (AKI 150.3% vs No AKI 82.1%; p=0.0002) and 24 h (AKI 222.3% vs 92.6%; p<0.0001) post CPB.

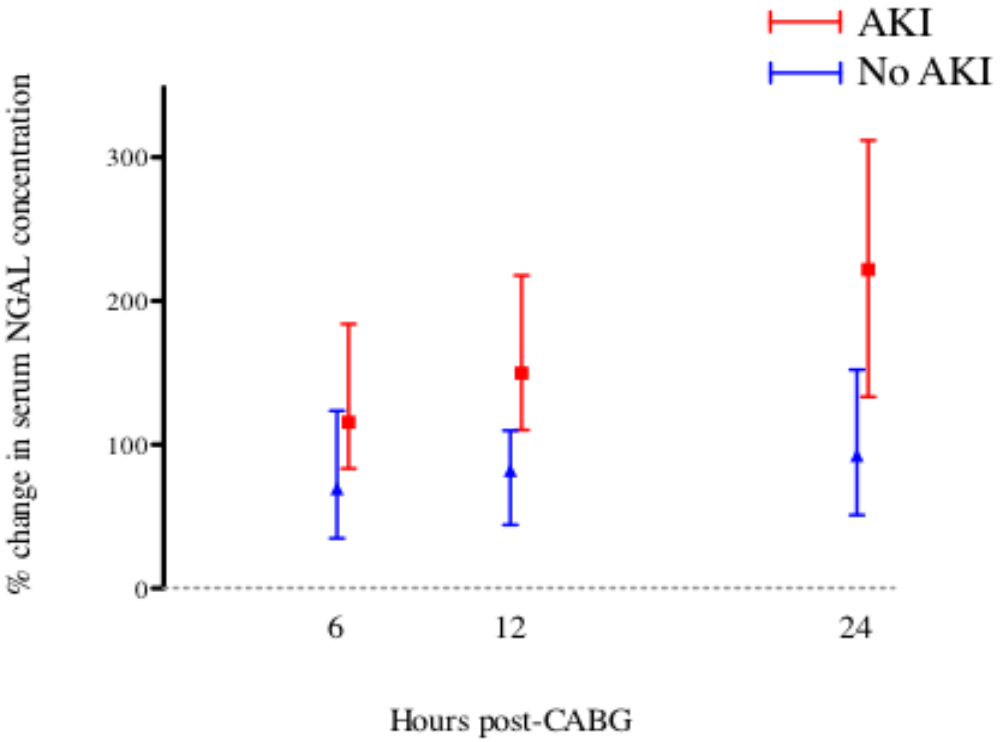


Figure 13 Median % increase in serum NGAL concentration over the first 24 h after cardiac surgery stratified by the presence or absence of AKI**. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values.** Percentage change in serum NGAL was significantly higher in the AKI group at 6 h (AKI 116.1% vs No AKI 69.5%; p=0.0046), 12 h (AKI 150.3% vs No AKI 82.1%; p=0.0002) and 24 h (AKI 222.3% vs 92.6%; p<0.0001) post CPB.

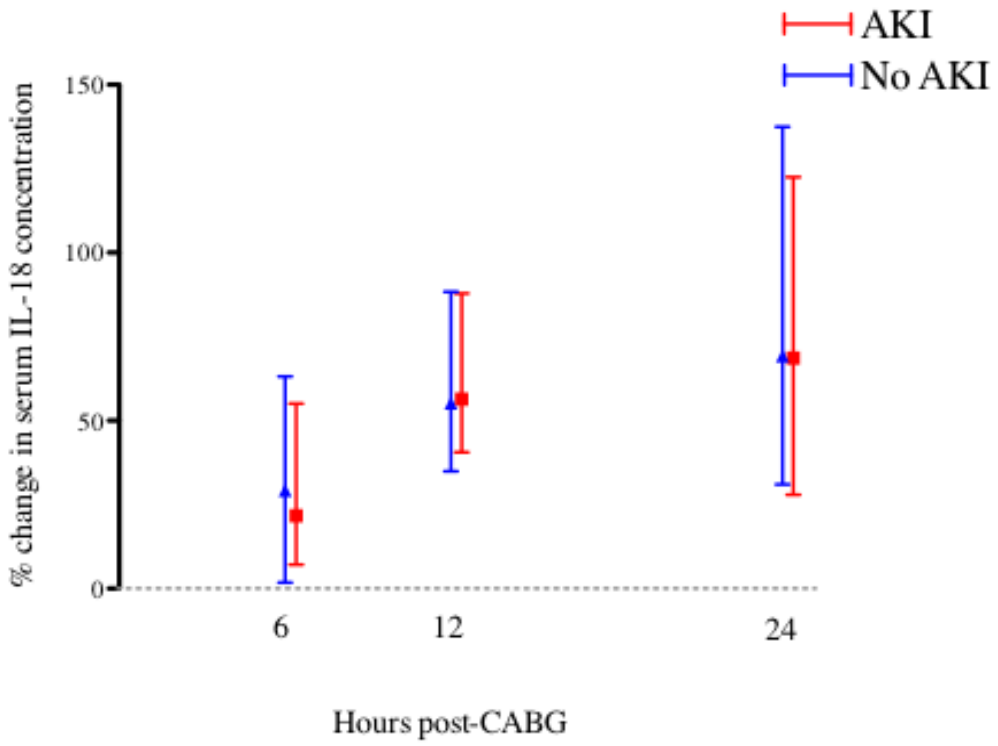


Figure 14 Median % increase in serum IL-18 concentration over the first 24 h after coronary surgery stratified by the presence or absence of AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. There was no significant difference in percentage change of serum IL-18 between the groups at any time point studied.

IL-18: There was no difference in pre-operative serum IL-18 level (AKI 18.5 [12.9 to 24.6] vs 20.0 [17.7 vs 21.1] ng/mL; p=0.531), or percentage change of serum IL-18 between the groups at any time point (**Figure 14**).

CyC: Median pre-operative serum CyC was not different between the groups (AKI 1372 [1218 to 1869] vs 1364 [1164 vs 1807] ng/mL; p=0.7518). Percentage change in serum CyC level was significantly higher in the patients with AKI at 12h (AKI 12.6% vs No AKI -10.9%; p=<0.0001) and 24h post CABG (AKI 42.1% vs No AKI -2.6%; p<0.0001) (**Figure 15**).

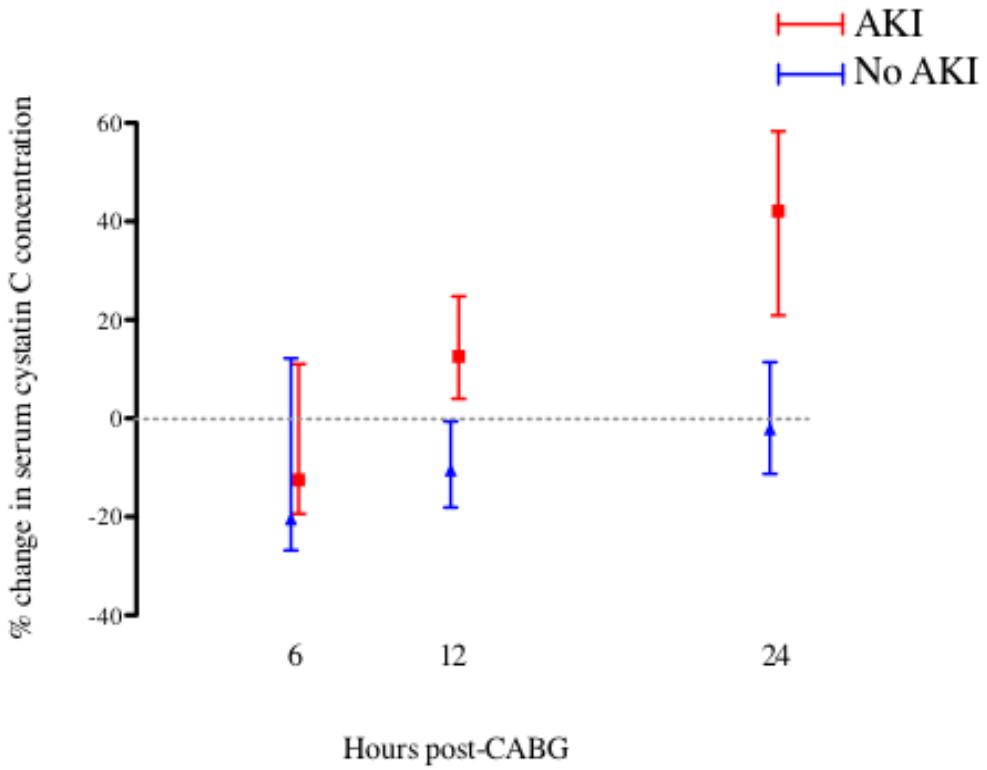


Figure 15 Median % increase in serum CyC concentration over the first 24 h after coronary surgery stratified by the presence or absence of AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. At 6 h post CABG serum CyC level have fallen from baseline in both groups. At 12 h (AKI 12.6% vs No AKI -10.9%; p=<0.0001) and 24h (AKI 42.1% vs No AKI -2.6%; p<0.0001) post-surgery percentage increase in serum CyC was significantly higher in the patients with AKI.

5.3.4 Urinary AKI biomarkers after CABG

NGAL: There was no significant difference in pre-operative urinary NGAL/urinary creatinine ratio between the groups (AKI 940.7 [361.3 - 2502.0] vs 593.5 [249.3 - 4241.0] ng/mg; p=0.7772). Median percentage increase in urinary NGAL/urinary creatinine ratio was higher 6, 12 and 24 h post-CABG in the AKI group. However, statistical significance was reached only at the 24 h time point (AKI 515% [162 – 2891] vs 149% [42 – 566]; p=0.0195) (**Figure 16**).

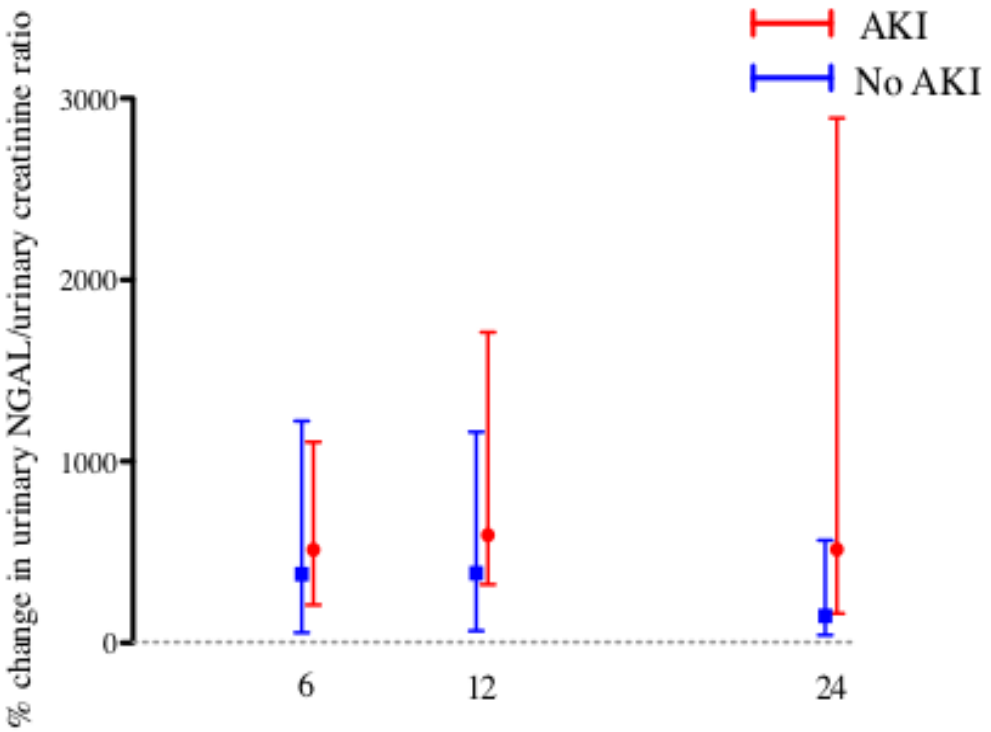


Figure 16 Median %increase in urinary NGAL/ urinary creatinine ratio over the first 24 h after coronary surgery stratified by the presence or absence of AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. Median percentage increase in urinary NGAL/urinary creatinine ratio was higher 6, 12 and 24 h post-CABG in the AKI group. However, statistical significance was reached only at the 24 h time point (AKI 515% [162 – 2891] vs 149% [42 – 566]; p=0.0195).

IL-18: There was no significant difference in pre-operative urinary IL-18/urinary creatinine ratio between the groups (AKI 1494.0 [398.7 – 2739.1] vs 942.4 [486.2 – 2107.0] ng/mg; p=0.6293). Post-operatively urinary IL-18/urinary creatinine ratios appear to fall from baseline levels in both groups. Paradoxically at both 12 h and 24 h post-CABG there was a greater reduction in urinary IL-18/urinary creatinine ratio in the AKI group compared with the no AKI group (**Figure 17**).

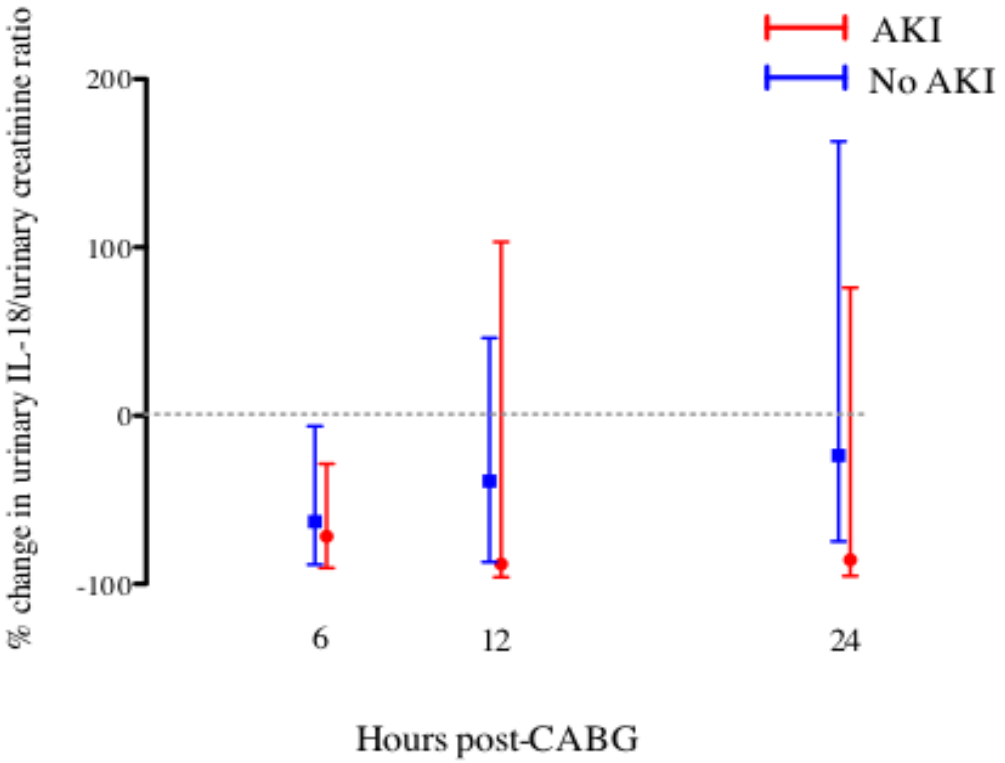


Figure 17 Median % change in urinary IL-18/ urinary creatinine ratio over the first 24 h after coronary surgery stratified by the presence of CSA-AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. There was no significant difference in percentage change of urinary IL-18/ urinary creatinine ratios between the groups at any time point studied

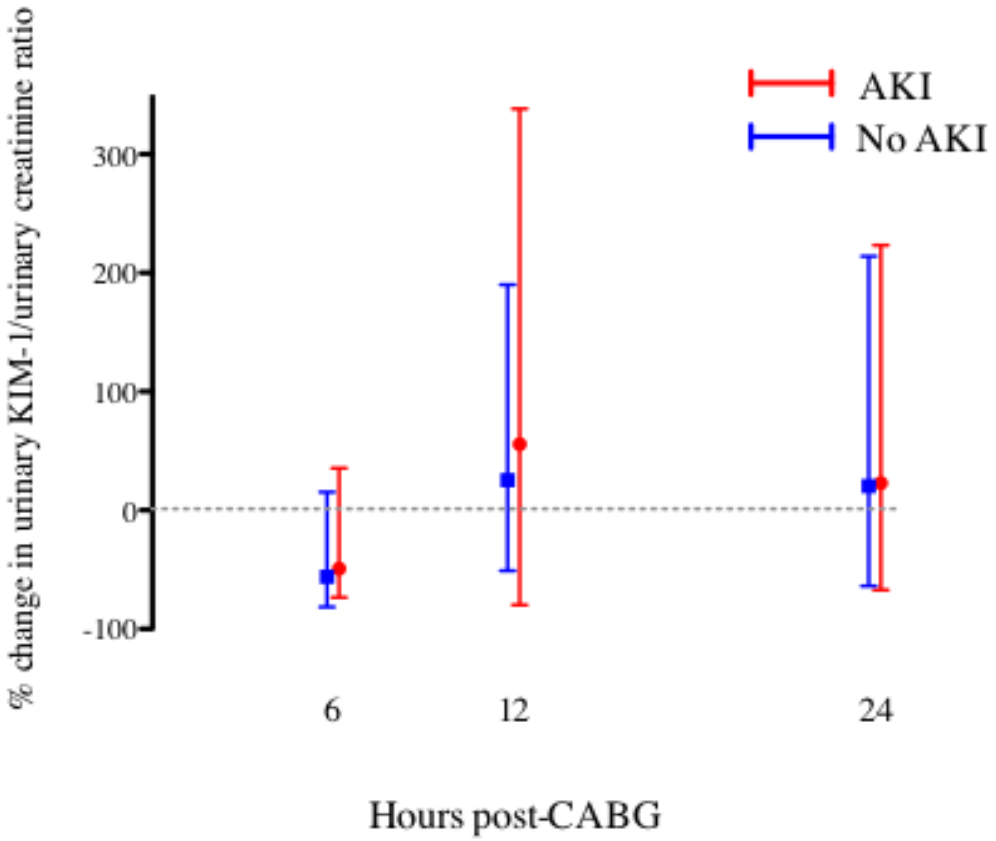


Figure 18 Median % change in urinary KIM-1/ urinary creatinine ratio over the first 24 h after coronary surgery stratified by the presence of CSA-AKI. The AKI group is displayed in red and the non-AKI group in blue. Error bars represent 25th, and 75th percentile values. There was no significant difference in percentage change of urinary KIM-1/ urinary creatinine ratios between the groups at any time point studied

KIM-1: There was no difference between the groups in urinary KIM-1/urinary creatinine ratio at baseline (AKI 54.3 [18.4 – 151.9] vs 60.1 [15.6 – 168.6] ng/mg; p=0.8287), or any subsequent time point (**Figure 18**).

5.3.5 Performance of biomarkers as predictors of AKI

Results of the ROC analysis are displayed in **Table 21**. The best performing serum biomarker for the prediction of AKI at all time points was serum CyC which displayed the highest AUC-ROC at 6 h (AUC-ROC 0.73 [0.60 – 0.86]), 12 h (AUC-ROC 0.86

[0.75-0.98]) and 24 h (AUC-ROC 0.94 [0.89-0.99]). Using a cut-off value of a 3.2% increase in concentration of cystatin C at 12 hours after surgery, cystatin C had a sensitivity of 81% and specificity of 59% for the prediction of AKI in our cohort. A cut-off value of a 17.8% increase in concentration of cystatin C at 24 hours after surgery gave a sensitivity of 88% and specificity of 87% for the prediction of AKI. Serum NGAL was a fair predictor at all time points with AUC at 6 h (AUC-ROC 0.71 [0.57 – 0.84]), 12 h (AUC-ROC 0.80 [0.70-0.90]) and 24 h (AUC-ROC 0.83 [0.74-0.93]). Serum IL-18 was poor predictor of AKI at all time points. The combination the two best performing serum biomarkers, NGAL and cystatin C, did not improve AUC-ROC for the predicting AKI within 12 h of surgery above that obtained with cystatin C alone (**Table 22**).

At the post-CABG time points studied, all of the urinary biomarkers performed poorly as predictors of AKI. The combination of urinary NGAL (the best performing urinary biomarker) with either serum NGAL or serum cystatin C or both was not associated with a better AUC-ROC for the early prediction of AKI than cystatin C alone.

Table 21 AKI biomarker test characteristics for the prediction of AKI

	Cut off (% biomarker increase)*	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	AUC-ROC (95% CI)
Serum Biomarkers						
NGAL 6 h	> 97	75	59	29	91	0.71 (0.57 – 0.84)
NGAL 12 h	> 110	81	76	43	95	0.80 (0.70 – 0.90)
NGAL 24 h	> 113	94	63	37	98	0.83 (0.74 – 0.93)
IL-18 6 h	> 28	81	13	18	75	0.51 (0.37 – 0.66)
IL-18 12 h	> 18	50	34	15	75	0.50 (0.35 – 0.66)
IL-18 24 h	> 97	31	43	11	73	0.52 (0.37 – 0.68)
CyC 6 h	> -16	69	67	32	90	0.74 (0.60 – 0.86)
CyC 12 h	> 3	81	59	59	95	0.86 (0.75 – 0.98)
CyC 24 h	> 18	88	87	61	97	0.94 (0.89 – 0.99)
Urine Biomarkers						
NGAL 6 h	> 111	94	33	24	96	0.56 (0.42 – 0.69)
NGAL 12 h	> 414	75	53	27	90	0.63 (0.49 – 0.77)
NGAL 24 h	> 143	81	50	27	92	0.69 (0.55 – 0.83)
IL-18 6 h	> -82	56	29	15	74	0.54 (0.39 – 0.70)
IL-18 12 h	> -82	38	17	9	55	0.67 (0.50 – 0.84)
IL-18 24 h	> -75	19	36	6	66	0.69 (0.54 – 0.83)
KIM-1 6 h	> -94	100	16	21	100	0.55 (0.41 – 0.70)
KIM-1 12 h	> -51	63	57	25	87	0.53 (0.36 – 0.70)
KIM-1 24 h	> -57	75	36	21	86	0.50 (0.35 – 0.66)

Optimal cut-off points for each biomarker were determined using the Youden index, which is calculated by adding sensitivity and specificity and subtracting 1 [243]. Biomarkers were dichotomized using the cut-offs to allow sensitivity, specificity, negative predictive value and positive predictive value for each biomarker to be calculated

Table 22 AKI biomarker panel characteristics for the prediction of AKI

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	AUC-ROC (95% CI)
6 h serum CyC/ serum NGAL	94	37	25	96	0.74 (0.60 – 0.87)
12 h serum CyC/ serum NGAL	100	39	27	100	0.85 (0.78 – 0.93)
6 h serum NGAL/ urine NGAL	100	19	22	100	0.59 (0.46 – 0.71)
12 h serum NGAL/ urine NGAL	100	37	27	100	0.65 (0.52 – 0.78)
6 h serum CyC / urine NGAL	94	23	22	94	0.56 (0.43 – 0.69)
12 h serum CyC / urine NGAL	100	41	28	100	0.64 (0.50 – 0.78)
6 h serum CyC / serum NGAL/ urine NGAL	100	10	20	100	0.58 (0.46 – 0.71)
12 h serum CyC / serum NGAL/ urine NGAL	100	21	25	100	0.67 (0.54 – 0.80)

5.4 Discussion

This study compared the ability of 3 serum and 3 urinary biomarkers to predict AKI after CABG in patients with CKD. Serum CyC was the best performing biomarker at all post-operative time points studied. The maximal diagnostic precision of CyC was at the 24 h post surgery time point when the AUC-ROC was 0.94. Serum NGAL also performed relatively well with a maximum AUC-ROC of 0.83 at the 24 h time point. Unfortunately serum IL-18 and all of the urinary biomarkers studied performed poorly as predictors of AKI in this cohort.

Although generally considered a surrogate marker of GFR, serum CyC has shown significant promise as a biomarker for predicting AKI [238]. It has never before been tested exclusively in a population of patients with pre-existing CKD. In this cohort by 12 h post CABG serum CyC levels were significantly higher in the patients that subsequently developed AKI when compared with patients that did not. At 24 h post CABG serum CyC could predict the subsequent development of AKI with a sensitivity of 88% and a specificity of 87%. AKI was not reliably detectable using sCr until 2 days after surgery. That serum CyC can accurately predict AKI at least 24 h earlier than the current gold-standard sCr clearly represents a significant advance. However diagnosis 12 – 24 h post CABG may still be too late to alter the course of AKI.

Both SCr and serum CyC are surrogate markers of GFR. They display a similar inverse linear relationship with GFR, so as GFR falls both sCr and serum CyC concentrations will increase. In this study AKI was defined in accordance with the consensus RIFLE criteria that emphasise post-operative change in sCr. As both sCr and serum CyC are related (by GFR), the impressive performance of serum CyC as a predictor of AKI in this cohort may simply be due the fact AKI is defined using sCr. Importantly elevation of serum CyC is apparent at a far earlier time point than sCr.

Serum NGAL also showed promise as a predictor of AKI. At 12 h post CABG the AUC-ROC for serum NGAL was 0.80 and this improved to 0.83 by the 24 h time point. Previous reports suggest that NGAL peaks in plasma/serum early after cardiac surgery, with its predictive accuracy for AKI at its highest within 2 - 6 h after separation from CPB [229, 230]. There was no 6 h NGAL peak in this cohort, rather serum NGAL levels steadily increased from baseline to 24 h post surgery. It should be noted that no

biomarker was sampled until 6 h post surgery. It is possible that serum NGAL levels peaked and fell prior to the 6 h sampling time point. Thus the performance of serum NGAL as an early predictor of AKI after CABG may have been underestimated. Serum NGAL has not been assessed before in cohort of patients with CKD. The release kinetics of NGAL in such a population remains undefined. Potentially patients with CKD may not be able to rapidly synthesize and secrete NGAL, and the early NGAL peak observed in non-CKD populations may simply not occur in patients with already diseased kidneys [235].

All of the urinary biomarkers studied performed poorly as predictors of AKI. There is no published data upon the predictive accuracy of either urinary IL-18 or KIM-1 in patients with CKD. The effect of renal function upon the diagnostic performance of urinary NGAL has been previously investigated, and consistent with these results, urinary NGAL was found to perform poorly as an AKI predictor in patients with an eGFR <60 mL/min [235]. Intact renal tubular cell function may be a prerequisite for rapid synthesis and secretion of urinary AKI biomarkers. Reduced renal tubular cell function in patients with CKD may render them unable to rapidly express these biomarkers in response to injury, and thus explain the poor predictive performance of these biomarkers observed in this study

Arguably AKI biomarkers in combination may perform better than any single biomarker. In this study the best performing serum and urine biomarkers were combined in order to evaluate their diagnostic performance within the first 12 h after CABG with the goal of accurate early prediction of AKI. Prediction of AKI early within the first 12 h after surgery is desirable for any potential intervention to be maximally effective. No combination biomarker 'panel' had a superior AUC-ROC to serum CyC alone for predicting AKI within the first 12 h post-CABG. AUC-ROC is essentially a trade off between sensitivity and specificity of a diagnostic test. However, relying upon AUC-ROC as a measure of test performance may lead to the under appreciation of a tests clinical utility. For example CyC had a sensitivity of 88% and a specificity of 87% for detecting AKI at 24 h. At this time AKI may be already established and refractory to intervention, thus the test is of limited utility. Combining serum and urine NGAL at 6 h after surgery had a sensitivity of 100% for detecting patients with CSA-AKI, albeit at the expense of a high false positive rate. Furthermore, the negative predictive value of this biomarker combination was 100%, which is clinically important, as it enables

patients that will not develop AKI to be reliably distinguished and spared inappropriate therapies at an early time point. Combination biomarker panels with high early sensitivity may be useful in clinical research, allowing the compilation of 'at-risk' study cohorts with a high-expected event rate. Evaluation of new AKI therapies in such high-risk cohorts is an attractive idea meaning low numbers of patients need to be recruited in order to adequately power prospective studies.

5.4.1 Strength and limitations of this study

To my knowledge this is the first study to evaluate novel candidate AKI biomarkers in a cohort of patients with CKD undergoing cardiac surgery. This is the major strength of the study. The lack of data upon biomarker performance in patients with CKD is an important gap in the current published literature. As patients with CKD now represent a significant proportion of patients undergoing cardiac surgery and form the majority of those who sustain post-operative AKI, specific research in this patient population was overdue.

The most important limitation of this study was the power. As only 86 patients were included in this study, and the frequency of AKI was only 19%, the study is underpowered to provide definitive conclusions on the performance of these biomarkers for predicting AKI in patients with CKD after CABG. Using only 16 examples of AKI in order to validate these biomarkers makes the generation of definitive statistical results challenging.

A second important limitation of this study is that it was a secondary analysis of the data collected in RIPC trial described in Chapter 4, and not a study specifically designed to examine the predictive value of AKI biomarkers. In the parent study troponin sampling time points were specified at 6, 12 and 24 h post-operatively in order to be comparable with similar published studies. For pragmatic reasons in this study biomarker samples were collected concurrently with troponin samples. However, the biomarker collection times represent an important potential limitation. Previous studies have suggested that serum/urine NGAL and urine IL-18 levels may peak early and fall within the first 6 h following cardiac surgery. Thus, by not sampling biomarkers until 6 h the predictive performance of these biomarkers may have been underestimated. Also it is possible that intervention in the parent study (namely RIPC) may have affected biomarker concentration. Although biomarkers levels are not different between the RIPC and

control groups in the parent study, it was inadequately powered to detect any small effect of RIPC upon these AKI biomarkers.

Finally, serum and urine biomarker concentrations were calculated using commercially available research ELISAs. There is inherent variability associated with the ELISA technique, which requires multiple complex steps. This may have affected the predictive performance of the biomarkers measured. Any variability is likely to be improved by increasing patient numbers and the number of sample repeats. Furthermore, there are now a number of available commercial platforms to measure each AKI biomarker, all with differing test characteristics. Comparison of biomarker performance between studies is limited by the lack of a standardized assay for each individual biomarker. The assays themselves may effect the results of the study. Notwithstanding these limitation both change in serum cystatin-C and serum NGAL performed well as predictors of CSA-AKI in this cohort.

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5.4.2 The future for AKI biomarker research

Combining AKI biomarkers with a clinical risk prediction model in order to more accurately define an individual's likelihood of developing CSA-AKI is an attractive proposition. To date a number of observational studies have identified several risk factors consistently associated with the development of AKI after cardiac surgery. As a result several models for predicting AKI after cardiac surgery have been developed[98, 100, 104-107]. The addition of AKI biomarkers to these models could further enhance the predictive accuracy of such models. Combining AKI biomarkers with a AKI risk prediction model was not possible in this cohort. It would have been inappropriate to apply any existing risk model to this study cohort, as no model has been validated in a CKD cohort, and generally these models predict the need for post-operative dialysis rather than RIFLE defined AKI. However, in the future it is likely that AKI biomarkers will have an important role in any clinical AKI risk model.

In general larger studies with consistent AKI definition, biomarker collection times, and biomarker measurement techniques are needed to develop standardised cut-offs for each biomarker. Such studies must include patients with CKD. As AKI is caused by multiple pathophysiological mechanisms it seems unlikely that a single biomarker would be sufficient for the accurate and consistent diagnosis of AKI. Although further research into individual biomarkers is important, the combination of biomarkers in multi-marker panels may prove the biggest step towards the accurate early diagnosis of AKI.

5.5 Conclusions

In this study serum CyC and serum NGAL were useful predictors of AKI in adults with CKD after CABG.

Chapter 6

6. Summary and Conclusions

6.1 Introduction

CKD is a major health problem that effects approximately 1 in 10 of the UK's adult population [9, 11]. Many patients with CKD develop accelerated, diffuse CAD that can be especially challenging to treat [244]. Myocardial revascularisation may improve survival in selected patients with CKD and multivessel CAD, and current international guidelines recommend CABG as the revascularisation modality of choice in this patient group [244]. Furthermore, patients with CKD and multivessel CAD appear to derive a survival advantage from CABG compared with PCI which is not present in patients with normal renal function [244]. For these reasons, the proportion of patients undergoing CABG who have CKD has increased steadily over the last 10 years, with patients with CKD now accounting for approximately one third of patients undergoing CABG [3, 150, 151, 163].

A particular problem for patients with CKD that undergo CABG is the development of post-operative AKI. Depending upon definition, AKI may complicate as many as one third of CABG operations [6, 99]. The development of AKI in this setting is associated with increased length of hospital stay, increased utilization of resources but most importantly it is associated with an increase in both short- and long-term post-operative mortality [5, 6, 106, 115]. Currently there are no prophylaxes or therapies proven to reduce the incidence of AKI post-CABG. The purpose of this thesis was to investigate strategies to reduce the incidence of AKI in patients with CKD undergoing cardiac surgery.

The specific aims of this thesis were:

1. In our local cardiac surgical cohort to evaluate,
    a. The effects of CKD upon outcome after CABG.

b. The importance of AKI after CABG.

2. To assess the potential for RIPC to reduce AKI and myocardial injury in patients with CKD undergoing CABG.
3. To investigate the diagnostic performance of serum and urine AKI biomarkers in a population of patients with CKD undergoing CABG.

6.2 Summary of thesis findings

Analysis of the Barts Health NHS Trust cardiac surgical dataset revealed some important findings. Firstly, patients with CKD (eGFR<60mL/min) are a group at particularly high risk of both early and late mortality following CABG. Notably these patients represent approximately one third of patients undergoing isolated coronary surgery but account for almost two thirds of all early mortality after CABG. Secondly, CKD was an important predictor of both short- and long-term post-operative mortality even after accounting for comorbidity that is commonly present in these patients. Thirdly, the relationship between mortality after CABG and renal dysfunction was non-linear. The risk of post-operative mortality increased steeply once eGFR fell below 60 mL/min. Finally, as expected the incidence of post-operative AKI was highest in patients with CKD.

My second analysis of the Barts Health NHS Trust cardiac surgical dataset highlighted the prognostic importance of AKI after CABG. In our local cardiac surgical population AKI was common, affecting 12% of patients after CABG. The development of AKI was associated with an increase in long-term mortality. Whether AKI has a causal relationship with subsequent mortality or its development simply reflects an increased burden of comorbidity and/or procedural complexity within a high-risk patient population already destined to do poorly after surgery is often debated. After using advanced statistical matching techniques to correct for baseline patient comorbidity and surgical factors AKI remained an important independent predictor of subsequent mortality in this population.

The pathophysiology of AKI following cardiac surgery is complex and incompletely understood. It is thought that renal IRI is an important component of a multifactorial renal insult that also includes a systemic inflammatory response to CPB, peri-operative haemodynamic instability, and/or peri-operative nephrotoxins. RIPC is a non-invasive

therapy that mitigates IRI and may modulate systemic inflammation in cardiac surgery. It is potentially a non-pharmacological strategy to reduce the incidence of AKI following cardiac surgery. RIPC has shown promise in reducing the incidence of AKI in patients undergoing major vascular surgery, coronary angiography and in cardiac surgery. RIPC has never before been evaluated specifically in a cohort of patients with CKD. Patients with CKD may accrue significant benefit from RIPC given the increased peri-operative risk they face. Unfortunately, in the randomized trial described in this thesis, RIPC despite its intuitive appeal did not reduce the incidence of AKI after CABG in patients with CKD.

Within the randomized RIPC trial serum and urine samples were collected for the analysis of a number of candidate AKI biomarkers. These biomarkers offer the potential for the early diagnosis of AKI at a time point when renal injury may be potentially modified. The performance of these biomarkers at predicting AKI in a population of patients with CKD undergoing CABG has never before been investigated. Serum CyC and serum NGAL performed impressively as predictors of AKI in patients with CKD. These biomarkers facilitated the diagnosis of AKI more than 24 hours before sCr.

6.3 Implications of this thesis

This thesis has helped to clarify the impact of pre-operative CKD upon outcomes after CABG. Understanding that patients with an eGFR<60mL/min are at such high risk of adverse outcomes following cardiac surgery is extremely important for cardiologists and cardiac surgeons when balancing the risks and benefits of CABG in different patient groups and when discussing the expected outcomes after surgery with their patients. This information applies to a significant proportion of patients undergoing CABG. In the unselected study population presented in this thesis, for example, approximately one third of the patients had CKD.

AKI is also a common problem following cardiac surgery. The development of this complication may identify patients with an increased risk of subsequent mortality following hospital discharge. Currently, there are no established prophylaxes or therapies proven to reduce the incidence nor the mortality associated with this condition. Hopefully this thesis and the publications arising from it will stimulate the closer post-discharge monitoring of patients that sustain AKI after cardiac surgery. In clinical

practice there is little specific follow-up of these patients. AKI at the time of cardiac surgery may result in ongoing progressive renal damage beyond the acute renal insult despite the normalization of sCr [114]. This is an important message for cardiologists, cardiac surgeons and renal physician responsible for the care of these patients following cardiac surgery. Although, future studies are needed to determine the optimal post-discharge follow-up and management of patients that sustain perioperative AKI, aggressive therapy to optimize control of cardiovascular risk factors should form the mainstay of the current management for these high-risk patients.

Unfortunately RIPC did not reduce the incidence of AKI in patients with CKD undergoing CABG. As with so much of the research into cardiac surgery associated AKI the randomized trial described in this thesis was small and underpowered. However, that RIPC failed to offer any meaningful cardiac or renal protection beyond conventional standard surgical and anaesthetic technique means that RIPC is unlikely to be readily adopted into clinical practice. Importantly, the AKI biomarkers measured in this study allowed the accurate early prediction of AKI in patients with CKD. These biomarkers will be central to our future understanding and management of CSA-AKI.

6.4 The future of cardiac surgery associated AKI

The acceptance of a consensus RIFLE definition of AKI following cardiac surgery represents an important advance within AKI research. It has allowed the comparison of clinical trials using a standardized end-point. However, the RIFLE criteria that emphasize percentage change in sCr and urine output for the diagnosis of AKI may in part be to blame for the lack of progress in developing new strategies to prevent or modify AKI. Both sCr and urine output are relatively insensitive and late markers of AKI, thus reliance upon the RIFLE criteria to define AKI will inevitably delay the recognition, and the institution of therapeutic interventions for AKI. Potentially AKI may be prevented if pharmacological interventions are initiated early. This is not possible relying upon the RIFLE definition of AKI.

Novel AKI biomarkers offer the promise of early accurate diagnosis of AKI following cardiac surgery. They will undoubtedly become central to any future definitions of AKI. Biomarker research is likely to lead to an evolution in our understanding of AKI. Combinations of biomarkers in multimarker panels, each with their own release

characteristics, will hopefully allow early identification of renal injury, stratification of the severity of injury, characterization of the location and/or aetiology of injury, provide prognostic information and allow the monitoring of response to therapy. Most importantly, early diagnosis of AKI will hopefully allow the institution of therapies that may modify renal injury at a time when the interventions may be effective. However, until novel therapeutic interventions that may modify the incidence or course of AKI become widely available, the early diagnosis of AKI is purely academic, as currently once established AKI cannot be modified.

The pre-operative estimation of risk of developing AKI after cardiac surgery is vital to individualize and optimize peri-operative care. Currently no model for predicting RIFLE defined AKI exists. The development of such a risk prediction model would be a major advance in this arena. Such a risk model that predicts lesser forms of AKI in patients with and without CKD would not only be of benefit to patients undergoing cardiac surgery, but would also be a powerful research tool for selecting patients for trials of renoprotective therapies. Any future risk model is likely to incorporate AKI biomarkers.

6.5 Conclusions

Firstly I must conclude that CKD is a particularly important risk factor for predicting adverse events following coronary revascularization. Secondly the development of an AKI at the time of revascularization portends an adverse long-term prognosis. Unfortunately, despite the intuitive appeal of RIPC it affords no meaningful cardiac or renal protection in patients with CKD undergoing cardiac surgery.

In the future better risk stratification will likely allow the prediction of patients that will develop AKI after cardiac surgery, and tailored care will likely contribute to improvements in renal outcomes. More importantly, updated definitions of AKI that emphasis novel AKI biomarkers will hopefully facilitate prompt AKI diagnosis, and will likely herald the development of new therapies to combat this important condition. Hopefully these changes, combined with larger randomized controlled trials, will lead to the improved outcomes for patents undergoing cardiac surgery.

Appendix

7. Appendix 1

7.1 Materials and Methods for Biomarker ELISA Assays

7.1.1 Standard solutions for all ELISA assays

1. Phosphate Buffered Saline (PBS)

Made by adding 1 sachets of Sigma PBS 7.4 (Cat P3813) to 1 L of H_20

Contains 137 mM Na Cl, 2.7 mm K Cl, 8.1 mM Na_2 HPO4, 1.5 mm KH_2PO_4, pH 7.2-7.4, 0.2μm filtered.

2. Reagent Buffer

Made by adding 5g of Fraction V Bovine Serum Albumin to 500 mL PBS

Contains 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered.

3. Wash Buffer

Made by adding 1 mL of neat Tween 20 to 2 L PBS

Contains 0.05% Tween 20 in PBS, pH 7.2-7.4

4. Substrate Solution (R and D Systems Catalogue # DY 999)

1:1 mixture of Colour Reagent A (H_2O_2) and Colour Reagent B (Tetramethylbenzidine)

5. Stop Solution (R and D Systems Catalogue # DY 994)

2N H_2SO_4

6. Streptavidin-HRP

Working solution made by diluting supplied Streptavidin-HRP solution (1 mL of streptavidin conjugated to horseradish-peroxidase) 1 in 200 in Reagent Diluent.

7.2 NGAL ELISA

Serum and urine NGAL levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (Human Lipocalin-2/NGAL Immunoassay; R&D Systems, Europe (Catalogue Number DY1757)

The kit contained:

Capture Antibody (rat anti-human Lipocalin-2)

Reconstituted by adding 1 mL of PBS to dry capture antibody to create diluted capture antibody with a concentration of 360 μg/mL. This diluted capture antibody solution was further diluted by adding 55 μL into 10 mL PBS to give working solution of 2 μg/mL.

Standards (recombinant human Lipocalin-2)

Reconstituted by adding 0.5 mL of reagent diluent to dry standard to create diluted NGAL standard with a concentration of 90 ng/mL. This standard solution was diluted 18 fold (50 μL added to 850 μL of reagent buffer to create working standard solution of 5000 pg/mL. The working standard solution was serially diluted to give standards with concentrations of: 5000, 2500, 1250, 625, 312.5, 156.25, 78.125 and 0 pg/mL

Detection Antibody (biotinylated goat anti-human Lipocalin-2)

Reconstituted by adding 1 mL of reagent dilutent to vial. This diluted detection antibody was further diluted by adding 55 μL into 10 mL reagent dilutent to give working solution of 100 ng/mL final concentration.

Serum and urine sample dilutions for NGAL ELISA

We identified that a dilution of serum samples of 1 in 200 with reagent buffer and of urine of 1 in 50 with reagent buffers as the most appropriate for our study population.

All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for NGAL concentration was 7.2% in our laboratory and this corresponds to that reported by the kit manufacturer.

Sample assays were repeated across several different plates on different days to ensure reproducibility of results although formal inter-assay variation was not calculated.

7.2.1 Assay Protocol

Plate Preparation

1. The working concentration of capture antibody is used to coat a 96-well microplate with 100 µL capture antibody solution/well. Plate then sealed and incubated overnight at room temperature.
2. Wash plate by aspirating each well with wash buffer, repeating the process twotimes for a total of three washes.
3. Block the plate by adding 300 µL of reagent diluent to each well. Incubate plates at room temperature for 1 h.
4. Repeat wash as in step 2.

Assay Procedure

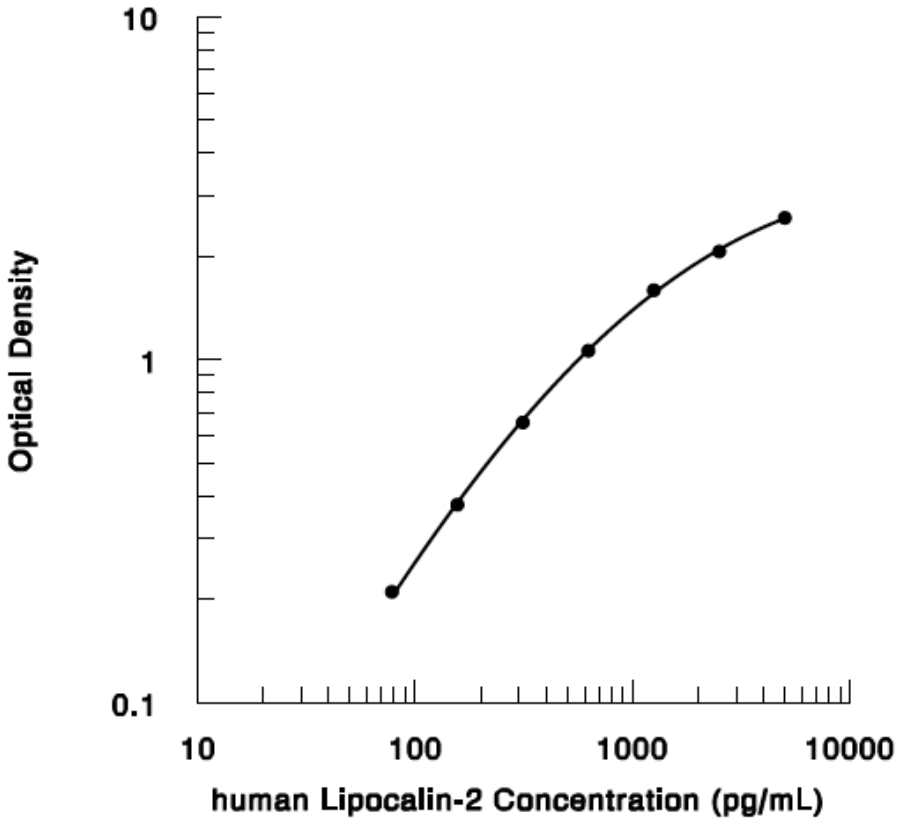
1. Add 100 µL of standards to first 16 wells of plate to create 'standard curve' for plate analysis.
2. Add 100 µL diluted serum or urine sample to remaining 80 wells of 96-well plate. Cover with an adhesive strip and incubate at room temperature for 2 h.
3. Repeat wash as in step 2 of plate preparation.
4. Add 100 µL of the working solution of detection antibody to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.
5. Repeat wash as in step 2 of plate preparation.
6. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.
7. Repeat wash as in step 2 of plate preparation.
8. Add 100 µL of substrate solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light.
9. Add 50 µL of stop solution to each well.
10. Determine the optical density of each well immediately using a microplate reader set to 450nm.

Calculation of results

All standards and samples were analysed in duplicate and the average taken as the final value.

A standard curve (**Figure 17**) was generated using Revelation microplate reading computer software (Dynex Technologies, Worthing, U.K), and allowed the derivation of sample NGAL concentration.

Figure 17 Standard curve for NGAL assay



7.3 IL-18 ELISA

Serum and urine IL-18 levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (Human IL-18 BPa Immunoassay; R&D Systems, Europe (Catalogue Number DY119)

The kit contained:

Capture Antibody (mouse anti-human CyC**)**

Reconstituted by adding 1 mL of PBS to dry capture antibody to create diluted capture antibody with a concentration of 720 μg/mL. This diluted capture antibody solution was further diluted by adding 55 μL into 10 mL PBS to give working solution of 4 μg/mL.

Standards (recombinant human IL-18)

Reconstituted by adding 0.5 mL of reagent diluent to dry standard to create diluted NGAL standard with a concentration of 250 ng/mL. This standard solution was diluted with reagent buffer to create working standard solution of 6000 pg/mL (21 μL standard diluted with 979 μL of reagent buffer). The working standard solution was serially diluted to give standards with concentrations of: 6000, 3000, 1500, 750, 375, 187.5, 93.75 and 0 pg/mL

Detection Antibody (biotinylated goat anti-human IL-18**)**

Reconstituted by adding 1 mL of reagent dilutent to vial. This diluted detection antibody was further diluted by adding 55 μL into 10 mL reagent dilutent to give working solution of 100 ng/mL final concentration.

Serum and urine sample dilutions for IL-18 ELISA

We identified that a dilution of serum samples of 1 in 40 in reagent buffer for both serum and urine as the most appropriate dilution for our study population.

All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for IL-18 concentration was 11.9% in our laboratory and this corresponds to that reported by the kit manufacturer.

Sample assays were repeated across several different plates on different days to ensure reproducibility of results although formal inter-assay variation was not calculated.

7.3.1 Assay Protocol

Plate Preparation

1. The working concentration of capture antibody is used to coat a 96-well microplate with 100 µL capture antibody solution/well. Plate then sealed and incubated overnight at room temperature.
2. Wash plate by aspirating each well with wash buffer, repeating the process twotimes for a total of three washes.
3. Block the plate by adding 300 µL of reagent diluent to each well. Incubate plates at room temperature for 1 h.
4. Repeat wash as in step 2.

Assay Procedure

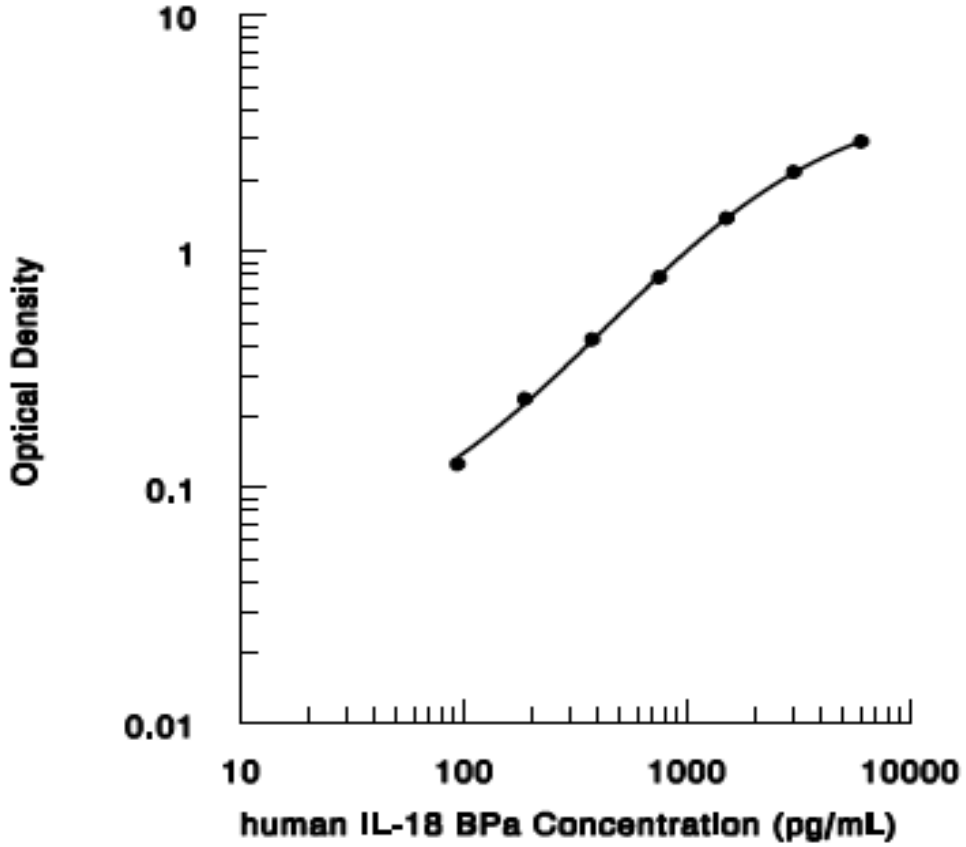
1. Add 100 µL of standards to first 16 wells of plate to create 'standard curve' for plate analysis.
2. Add 100 µL diluted serum or urine sample to remaining 80 wells of 96-well plate. Cover with an adhesive strip and incubate at room temperature for 2 h.
3. Repeat wash as in step 2 of plate preparation.
4. Add 100 µL of the working solution of detection antibody to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.
5. Repeat wash as in step 2 of plate preparation.
6. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.
7. Repeat wash as in step 2 of plate preparation.
8. Add 100 µL of substrate solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light.
9. Add 50 µL of stop solution to each well.
10. Determine the optical density of each well immediately using a microplate reader set to 450nm.

Calculation of results

All standards and samples were analysed in duplicate and the average taken as the final value.

A standard curve (**Figure 18**) was generated using Revelation microplate reading computer software (Dynex Technologies, Worthing, U.K), and allowed the derivation of sample IL-18 concentration.

Figure 18 Standard curve for IL-18 assay



7.4 CYC ELISA

Serum CyC levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (Human CyC Immunoassay; R&D Systems, Europe (Catalogue Number DY1196)

The kit contained:

Capture Antibody (mouse anti-human CyC)

Reconstituted by adding 1 mL of PBS to dry capture antibody to create diluted capture antibody with a concentration of 720 μg/mL. This diluted capture antibody solution was further diluted by adding 55 μL into 10 mL PBS to give working solution of 4 μg/mL.

Standards (recombinant human CyC)

Reconstituted by adding 0.5 mL of reagent diluent to dry standard to create diluted CyC standard with a concentration of 95 ng/mL. This standard solution was diluted 47.5 fold (21 μL added to 979 μL of reagent buffer to create working standard solution of 2000 pg/mL. The working standard solution was serially diluted to give standards with concentrations of: 2000, 1000, 500, 250, 125, and 0 pg/mL

Detection Antibody (biotinylated mouse anti-human CyC)

Reconstituted by adding 1 mL of reagent dilutent to vial. This diluted detection antibody was further diluted by adding 55 μL into 11 mL reagent dilutent to give working solution of 250 ng/mL final concentration.

Serum sample dilution for CyC ELISA

We identified that a dilution of serum samples of 1 in 1250 in reagent buffer as the most appropriate dilution for our study population.

All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for CyC concentration was 5.1% in our laboratory and this corresponds to that reported by the kit manufacturer.

Sample assays were repeated across several different plates on different days to ensure reproducibility of results although formal inter-assay variation was not calculated.

7.4.1 Assay Protocol

Plate Preparation

1. The working concentration of capture antibody is used to coat a 96-well microplate with 100 µL capture antibody solution/well. Plate then sealed and incubated overnight at room temperature.
2. Wash plate by aspirating each well with wash buffer, repeating the process twotimes for a total of three washes.
3. Block the plate by adding 300 µL of reagent diluent to each well. Incubate plates at room temperature for 1 h.
4. Repeat wash as in step 2.

Assay Procedure

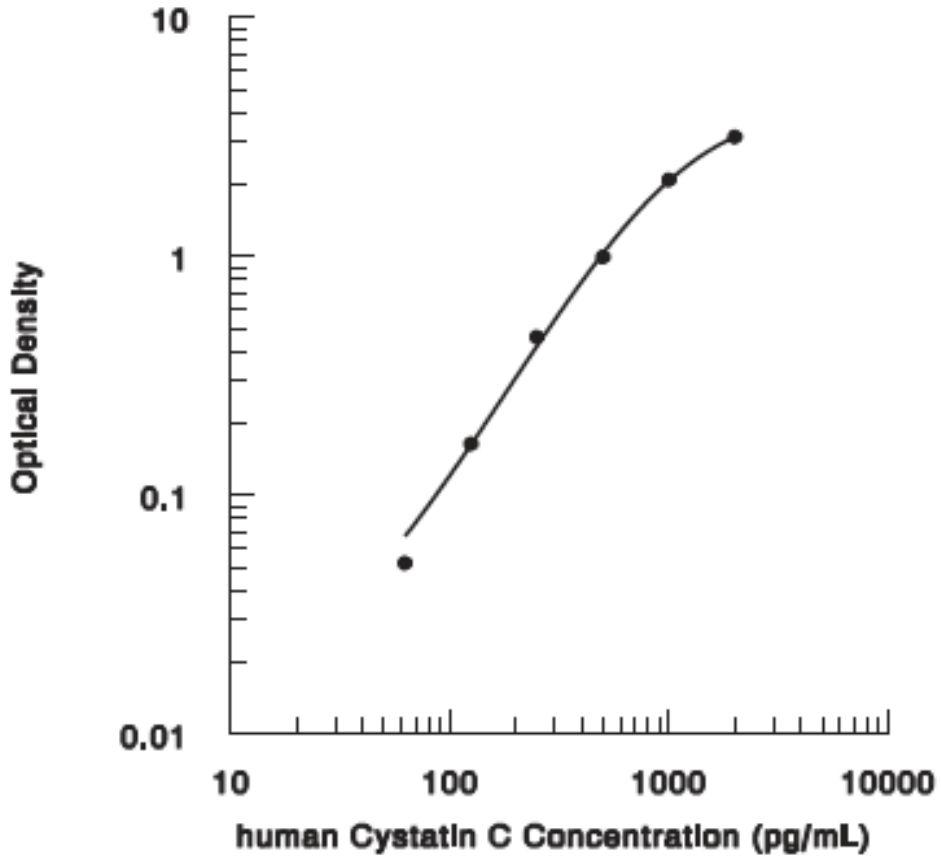
1. Add 100 µL of standards to first 16 wells of plate to create 'standard curve' for plate analysis.
2. Add 100 µL diluted serum or urine sample to remaining 80 wells of 96-well plate. Cover with an adhesive strip and incubate at room temperature for 2 h.
3. Repeat wash as in step 2 of plate preparation.
4. Add 100 µL of the working solution of detection antibody to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.
5. Repeat wash as in step 2 of plate preparation.
6. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.
7. Repeat wash as in step 2 of plate preparation.
8. Add 100 µL of substrate solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light.
9. Add 50 µL of stop solution to each well.
10. Determine the optical density of each well immediately using a microplate reader set to 450nm.

Calculation of results

All standards and samples were analysed in duplicate and the average taken as the final value.

A standard curve (**Figure 19**) was generated using Revelation microplate reading computer software (Dynex Technologies, Worthing, U.K), and allowed the derivation of sample CyC concentration.

Figure 19 Standard curve for CyC assay



7.5 KIM-1 ELISA

Urinary KIM-1 levels were detected by using a commercially available enzyme-linked immunosorbent assay kit (TIM-1/KIM-1/HAVCR Immunoassay; R&D Systems, Europe (Catalogue Number DY1750)

The kit contained:

Capture Antibody (goat anti-human TIM-1)

Reconstituted by adding 1 mL of PBS to dry capture antibody to create diluted capture antibody with a concentration of 72 μg/mL. This diluted capture antibody solution was further diluted by adding 55 μL into 10 mL PBS to give working solution of 0.4 μg/mL.

Standards (recombinant human TIM-1)

Reconstituted by adding 0.5 mL of reagent diluent to dry standard to create diluted TIM-1 standard with a concentration of 105 ng/mL. This standard solution was diluted 52.5 fold (19 μL added to 981 μL of reagent buffer to create working standard solution of 2000 pg/mL. The working standard solution was serially diluted to give standards with concentrations of: 2000, 1000, 500, 250, 125, 62.5, 31.25 and 0 pg/mL

Detection Antibody (biotinylated goat anti-human TIM-1)

Reconstituted by adding 1 mL of reagent dilutent to vial. This diluted detection antibody was further diluted by adding 55 μL into 11 mL reagent dilutent to give working solution of 400 ng/mL final concentration.

Serum sample dilution for KIM-1 ELISA

We identified that a dilution of urine samples of 1 in 2.5 in reagent buffer as the most appropriate dilution for our study population.

All samples from an individual patient were measured in a single assay. The co-efficient of variation for intra-assay reproducibility for KIM-1 concentration was 4.8% in our laboratory and this corresponds to that reported by the kit manufacturer.

Sample assays were repeated across several different plates on different days to ensure reproducibility of results although formal inter-assay variation was not calculated.

7.5.1 Assay Protocol

Plate Preparation

1. The working concentration of capture antibody is used to coat a 96-well microplate with 100 µL capture antibody solution/well. Plate then sealed and incubated overnight at room temperature.
2. Wash plate by aspirating each well with wash buffer, repeating the process twotimes for a total of three washes.
3. Block the plate by adding 300 µL of reagent diluent to each well. Incubate plates at room temperature for 1 h.
4. Repeat wash as in step 2.

Assay Procedure

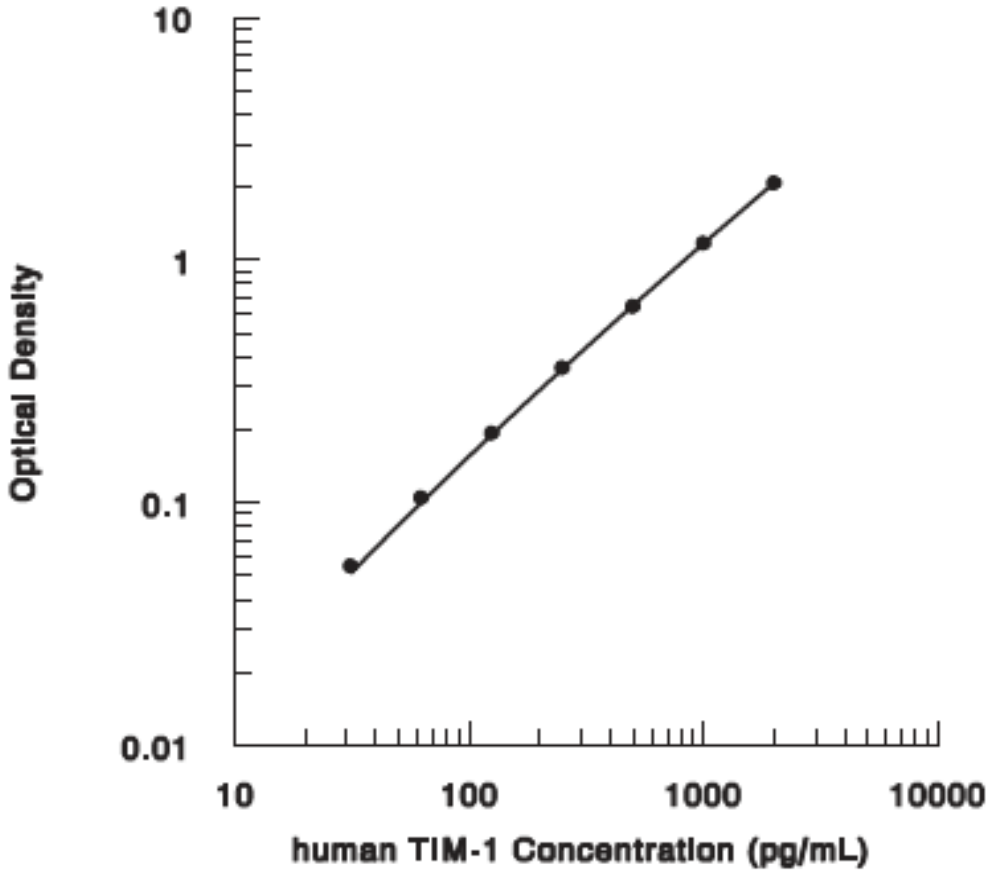
1. Add 100 µL of standards to first 16 wells of plate to create 'standard curve' for plate analysis.
2. Add 100 µL diluted serum or urine sample to remaining 80 wells of 96-well plate. Cover with an adhesive strip and incubate at room temperature for 2 h.
3. Repeat wash as in step 2 of plate preparation.
4. Add 100 µL of the working solution of detection antibody to each well. Cover with a new adhesive strip and incubate for 2 h at room temperature.
5. Repeat wash as in step 2 of plate preparation.
6. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 min at room temperature. Avoid placing the plate in direct light.
7. Repeat wash as in step 2 of plate preparation.
8. Add 100 µL of substrate solution to each well. Incubate for 20 min at room temperature. Avoid placing the plate in direct light.
9. Add 50 µL of stop solution to each well.
10. Determine the optical density of each well immediately using a microplate reader set to 450nm.

Calculation of results

All standards and samples were analysed in duplicate and the average taken as the final value.

A standard curve (**Figure 20**) was generated using Revelation microplate reading computer software (Dynex Technologies, Worthing, U.K), and allowed the derivation of sample KIM-1 concentration.

Figure 20 Standard curve for CyC assay



7.6 Urinary creatinine assay

Creatinine Assay Kit; Abcam, Cambridge, UK

Catalogue No. ab65340

Kit Components

Creatinine assay buffer	25mLs	
Creatinine Probe (in DMSO)	200µL	
Creatinase	1 vial	Reconstitute with 220 µL assay buffer
Creatininase	1 vial	Reconstitute with 220 µL assay buffer
Creatinine enzyme mix	1 vial	Reconstitute with 220 µL assay buffer
10 µmol Creatinine	1 vial	Reconstitute with 100 µL H_20

7.6.1 Assay Protocol

Standard Preparation

1. Mix 10 µL of creatinine standard with 990 µL of creatinine assay buffer to generate 1 nmol/L standard working solution.
2. Dilute this standard working solution 10 fold (100 µL standard with 900 µL assay buffer).
3. Add 0, 2, 4, 6, 8, 10 µL of diluted standard working solution to 6 consecutive wells of a 96-well microplate. Then add 50, 48, 44, 42, 40 µL of assay buffer to the wells to bring the volume of each well to 50 µL.

Sample preparation: add 50 µL of samples to each well.

Prepare reaction mix: Each well should contain 42 µL assay buffer, 2 µL creatinase, 2 µL creatininase, 2 µL creatinine enzyme mix and 0.4 µL creatinine probe. Therefore for the 96 well plate mix 4032 µL assay buffer, 196 µL creatinase, 196 µL creatininase, 196 µL creatinine enzyme mix and 38.4 µL creatinine probe and add 48.4 µL of this reaction mix to each standard and sample well. Incubate at 37ºC for 1 h.

Read the plate in a fluorescence reader with Ex/Em=538/587 nm. Sample creatinine concentrations are determined from a standard curve calculated from standard assays.

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